Official Organ of the Society for the Study of Development and Growth

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HEIGHT, WEIGHT AND BODY INDEX OF SCHOOL CHILDREN. A CORRELATION STUDY.

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Medical Research Committee, National Advisory Council for Physical Education, Pretoria, Union of South Africa

In the following are presented the results of an intensive study of growth, health, absenteeism records, physical efficiency, scholastic performances and social background of 114 white school children of between 6-7 years of age. This paper is concerned with an analysis of the significance upon the children's physical, health and social status of three anthropometric determinators, namely body length, body weight and body bulk.

METHODS

Height and Weight: Height and weight measurements were taken.

Clinical Status: A clinical survey was conducted by Professor Kloppers of the Department of Medicine of the University of Pretoria. His method has been described (1) and Table I indicates its scope.

Dental Survey: A dental survey was conducted by Dr. Fraser of the Municipal Dental Clinic in Johannesburg. Presence or absence respectively of cavities or extracted teeth, number of fillings and retained roots were ascertained for every child. Regularity of dental patterns, presence or absence respectively of dentures, mal-occlusion, incidence of caries, saveable, unsaveable, filled and missing teeth, retained roots, gingival condition and pathological occurrences other than the ones referred to above were noted (2).

Absenteeism: Records were kept of absenteeism from school.

Physical Efficiency: Three physical efficiency tests were applied, namely 100 and 600 yards running and putting the 12 lb. shot. The results obtained in these tests were evaluated on the South African standard grids for boys and girls (3).

Scholastic Achievements: I.Q. tests were applied but the children's scholastic achievements were assessed from their class performances.

We hold that basically intelligence is determined by an inherent quality, but that it can be measured only in respect of performances in fields in which acquired concepts are handled. Means were calculated from the children's class marks and individual records established on a percentage basis. The results were evaluated separately for boys and girls (4).

Social Background: Professor Gray of the Department of Social Sciences of the University of the Witwatersrand organized a survey of the social and economic status of the homes of the children. From the data thus collected we have calculated monthly income averages per head of member of household, and the number of persons with whom each child shared its bedroom (5).

Statistical Evaluation: All the methods of investigation employed in this survey render quantitative expression of the results possible. Our material was statistically evaluated in accordance with accepted procedures and small sample tests were applied if considered necessary and appropriate. Means, standard deviations and standard errors were ascertained and Student's t-test and Pearson's chi-squared analysis used to ascertain significance of difference (6). Separate calculations for boys and girls were made in respect of such factors only for which an analysis of intersexual differences has revealed the existence of categorical discrepancies. Wherever percentage figures had to be compared, significance of difference was judged by consistency of results and magnitude of divergencies wherever they were encountered.

Determinator I: Body Length

The entire range of measurements obtained from a sample of 114 children (40.5 ins.-51.63 ins.) was divided into three equal sectors and boys and girls were grouped in accordance with their heights. Since the mean ages of the children differed slightly in that the shortest children happened to be a few months younger than the tallest, we tested for significance of the differences in terms of standard means of body length calculated for the different ages of the three subgroups. Standard means were determined from our South African height grids. The differences of the means for the three groups were found to be highly significant.

TABLE I

HEIGHT, WEIGHT AND BODY INDEX

Determinator:	Height (in ins.)									Weight (in lbs.)									Body Index								
	Height			Weight			Body Index			Height			Weight			Body Index			Height			Weight			Body Index		
Sub-Group:	Short	Med.	Tall	Light	Med.	Heavy	Low	Med.	High	Short	Med.	Tall	Light	Med.	Heavy	Low	Med.	High	Short	Med.	Tall	Light	Med.	Heavy	Low	Med.	High
No. of																											
Boys	15	15	17	15	15	17	15	15	17	13	15	19	13	15	19	13	15	19	12	19	16	12	19	16	12	19	16
Girls	26	17	24	26	17	24	26	17	24	23	22	22	23	22	22	23	22	22	27	14	26	27	14	26	27	14	26
Means for																											
Boys	43.8	45.2	48.0	40.8	43.7	49.2	1.495	1.506	1.505	44.2	45.0	47.4	39.2	43.3	49.7	1.411	1.511	1.558	45.9	45.6	46.0	41.8	43.5	48.5	1.390	1.474	1.620
Girls	43.4	45.2	47.6	40.2	42.2	46.9	1.506	1.467	1.458	44.0	45.0	47.0	38.4	42.9	48.8	1.410	1.494	1.538	45.4	45.3	45.3	40.2	43.4	46.1	1.369	1.486	1.589

Weight and Body Index: There were significant differences in regard to body weight and body index of the three groups, the smallest children being much lighter and having much less bulk for a given height than had the medium and tall groups. This applied to the boys as well as to the girls.

Health: With regard to the results of the clinical survey for the entire sample of which the material used in this study forms a portion, we refer to a report previously published by Jokl and Kloppers (7).

No differences of significance were found in respect of the condition of the children's skins; the incidence of scabies, impetigo as well as of cutaneous manifestations of nutritional deficiencies was about the same in all three samples. Sub-skin tissue was "best" in the short group (51.4% marked as "good" as against 22.2% in the medium and 8.6% in the tall). The position was similar in regard to the state of the children's musculature which was considered "good" in 35.1% of the short as compared with 25.9% of the middle and 17.1% of the tall group. No differences were found between the short and the tall in respect of posture. The incidence of "fatigued" posture, round shoulders, kyphosis, scoliosis, lordosis and flat feet was about equal in the three sub-groups. The same statement applies to the condition of the children's eyes, ears, nose and lips. Cheilosis (cracked lips) and angular stomatitis (inflammation around the angles of the mouth), i.e., important indicators of nutritional deficiency, were equally distributed among the short, the medium sized and the tall children.

As has been previously reported by Fraser (8), there was a high incidence of caries of the teeth among the children. But our figures do not indicate any differences of significance in this respect between the three sub-groups. The same statement applies to the percentage incidence of spongy gums, of enlarged and septic tonsils and of enlarged neck glands. Among the tall children there was, however, a significantly higher incidence of flat as against well rounded chests than was found among the small ones, the corresponding percentage figures for the three groups being 51.4, 44.4 and 37.8.

Absenteeism: On the whole the children missed about 10% of the total possible attendance maximum. The short children attended school at a slightly more regular rate and although statistically the differences are not significant for our sample, the consistence with

which the short children topped the list during all four terms of the year suggests the possibility of significance.

Physical Efficiency: The tallest boys as well as the tallest girls showed the best physical performances. The differences between the shortest and tallest performers were considerable, amounting to 1.9 and 3.2 seconds respectively in the 100 yards race for boys and girls, to 21.2 and 27.4 seconds in the 600 yards race, and to 9 ins. and 10 ins. in the shotput. These differences are equivalent to a standard growth progress at this age of mean performance values of between one and two years.

Scholastic Performances: No significant differences were found between the scholastic performances of the three groups.

Social Background: No significant differences were found in respect of the number of persons with whom the children shared their bedroom, as well as of the monthly income average per head of household.

Determinator II: Weight

The entire range of weight measurements from a sample of 114 children (31 lbs.-56 lbs.) was divided into three equal sectors and boys and girls were grouped accordingly. The differences for the means of the three groups were found to be highly significant in absolute terms as well as in terms of standard means for the respective ages.

Height and Body Index: In all groups the heavier children were taller and had a higher body index, i.e., they were bulkier for their height.

Health: As was to be expected ex hypothesi there was a preponderance of linear or asthenic body types among the light (23.3% as against 36.4% for the medium and 50% for the heavy) children, who also showed the smallest number of normal elastic skins (10% as against 15.2% for the medium and 19.4% for the heavy group) and a slightly higher incidence of dry (86.7% as against 75.8% and 69.4%) and of rough skin (46.6% as against 39.4% and 41.7%) and of dermatitis (10% as against 3% and 2.8%) than did the other groups. The differences are not great but they are consistent and therefore suggestive of significance. Scabies (5.6% in the heavy as against 21.2% for the medium and 16.7% for the light) and impetigo (19.4% in the heavy as against 21.2% for the medium and 26.7% for the

TABLE II
CLINICAL SURVEY
(Figures indicate Percentage Incidence)

Determinator:	Height			Weight			Body Index		
Sub-Group:	Short	Med.	Tall	Light	Med.	Heavy	Low	Med.	High
No. (Boys	13	14	15	11	13	18	10	17	15
of (Girls	24	13	20	19	20	18	18	16	23
TOTAL	37	27	35	30	33	36	28	33	38
BUILD:									
Linear	29.7	37.0	68.6	23.3	36.4	50.0	71.4	60.6	15.8
Intermediate	35.1	44.4	25.7	33.3	33.3	30.6	25.0	30.3	42.1
Lateral	35.1	18.5	5.7	13.3	30.3	19.4	3.6	10.0	42.1
SKIN:									
Elastic	16.2	18.5	11.4	10.0	15.2	19.4	10.7	10.0	23.7
Dry	75.7	74.1	85.7	86.7	75.8	69.4	85.7	81.8	65.8
Rough	35.1	44.4	40.0	46.6	39.4	41.7	46.4	51.5	28.9
Dermatitis	8.1	7.4	2.9	10.0	3.0	2.8	14.3	10.0	—
Phrynoderma	—	—	2.9	3.3	—	2.8	—	—	2.6
"Sulphur Granules"	—	—	—	—	—	—	—	—	—
Scabies	13.5	14.8	17.1	16.7	21.2	5.6	21.4	18.2	5.3
Impetigo	21.6	29.6	20.0	26.7	21.2	19.4	17.9	27.3	15.8
"Mosaic"	5.4	3.7	—	—	9.1	2.8	—	6.1	7.9
Other	8.1	—	—	6.7	3.0	—	7.1	—	2.6
SUBSKIN TISSUE:									
Good	51.4	22.2	8.6	23.3	36.4	25.0	7.1	18.2	52.6
Average	18.9	44.4	25.7	23.3	24.2	36.1	10.7	39.4	31.6
Poor	29.7	33.3	65.7	53.3	39.4	38.9	82.1	42.4	15.8
MUSCULATURE:									
Good	35.1	25.9	17.1	20.0	27.3	30.6	10.7	18.2	44.7
Average	29.7	37.0	20.0	20.0	30.3	27.8	—	30.3	39.5
Poor	35.1	33.3	62.9	56.7	42.4	41.7	85.7	51.5	15.8
Tender	—	3.7	—	3.3	—	—	3.6	—	—
POSTURE:									
Satisfactory	24.3	18.5	22.9	13.3	30.3	19.4	10.7	18.2	31.6
Fatigued	10.8	18.5	20.0	13.3	12.1	19.4	14.3	24.2	7.9
Round Shoulders	54.1	74.1	60.0	73.3	57.6	61.1	78.6	63.6	52.6
Kyphosis	16.2	33.3	17.1	20.0	24.2	19.4	17.9	24.2	21.1
Scoliosis	5.4	—	14.3	6.7	3.0	11.1	14.3	12.1	—
Lordosis	51.4	48.1	45.7	60.0	39.4	50.0	53.6	54.5	39.5
Flat Feet	40.5	40.7	28.6	33.3	42.4	33.3	35.7	39.4	34.2
Knock-knees	2.7	—	2.9	—	—	5.6	—	3.0	2.6
Bow-legs	—	—	—	—	—	—	—	—	—
Enlarged Wrists	—	—	—	—	—	—	—	—	—
Other	2.7	—	—	—	3.0	—	—	—	2.6
EYES:									
N. A. D.	91.9	92.6	94.3	93.3	97.0	88.9	92.9	93.9	92.1
Discharge	5.4	7.4	5.7	6.7	3.0	8.3	7.1	6.1	5.3
Conjunctivitis	5.4	—	—	3.3	—	2.8	3.6	—	2.6
Blepharitis	—	—	—	—	—	—	—	—	—
Bitot's Spots	—	—	—	—	—	—	—	—	—
Corneal Vascularization	—	—	—	—	—	—	—	—	—
Blind	—	—	—	—	—	—	—	—	—
Other	—	—	—	—	—	—	—	—	—

TABLE II (*continued*)

Determinator:	Height			Weight			Body Index		
Sub-Group:	Short	Med.	Tall	Light	Med.	Heavy	Low	Med.	High
No. (Boys	13	14	15	11	13	18	10	17	15
of (Girls	24	13	20	19	20	18	18	16	23
TOTAL	37	27	35	30	33	36	28	33	38
EARS:									
N. A. D.	97.3	100.0	100.0	96.7	100.0	100.0	96.4	100.0	100.0
Discharge	2.7	—	—	3.3	—	—	3.6	—	—
Deaf	—	—	—	—	—	—	—	—	—
Other	—	—	—	—	—	—	—	—	—
NOSE:									
N. A. D.	75.7	81.4	68.6	73.3	81.8	69.4	75.0	69.7	78.9
Nasolabial Fissures	5.4	—	—	10.0	—	—	3.6	3.0	—
Other	18.9	18.5	31.4	16.7	18.2	30.6	21.4	27.3	21.1
LIPS:									
Cheilosis	45.9	40.7	57.1	43.3	36.4	52.8	53.6	45.5	44.7
Angular Stomatitis	18.9	29.6	17.1	20.0	21.2	22.2	21.4	21.2	18.4
TONGUE:									
Magenta Glossitis	—	3.7	—	—	3.0	—	—	3.0	—
Atrophic Glossitis	2.7	7.4	—	3.3	3.0	2.8	3.6	—	5.3
Scarlet Red Stomatitis	—	—	—	—	—	—	—	—	—
TEETH:									
N. A. D.	16.2	25.9	17.1	20.0	15.2	25.0	14.3	15.2	28.9
Slight Caries	59.5	59.3	60.0	63.3	63.6	55.6	67.9	63.6	50.0
Severe Caries	24.3	14.8	22.9	16.7	21.2	19.4	17.9	21.2	21.1
Other	—	—	—	—	—	—	—	—	—
GUMS:									
N. A. D.	56.8	74.1	65.7	56.7	66.7	61.1	57.1	60.6	65.8
Spongy	43.2	25.9	34.3	43.3	33.3	38.9	42.9	39.4	34.2
Septic	—	—	—	—	—	—	—	—	—
Other	—	—	—	—	—	—	—	—	—
PHARYNX:									
Scarlet Red	5.4	11.1	5.7	10.0	6.0	5.6	—	15.2	5.3
Tonsils Enlarged	27.0	33.3	34.3	33.3	24.2	41.7	39.3	27.3	28.9
Enlarged and Septic	35.1	37.0	34.3	33.3	33.3	36.1	39.3	33.3	31.6
Other	—	—	—	—	—	—	—	—	—
NECK GLANDS:									
N. A. D.	21.6	29.6	28.6	10.0	39.4	22.2	14.3	27.3	28.9
Enlarged	78.4	70.4	71.4	90.0	60.6	77.8	85.7	72.7	71.1
THYROID:									
N. A. D.	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Enlarged	—	—	—	—	—	—	—	—	—
CHEST:									
N. A. D.	62.2	55.6	48.6	50.0	63.6	55.6	39.3	60.6	63.2
Flat	37.8	44.4	51.4	50.0	36.4	44.4	60.7	39.4	36.8
Rickety	—	—	—	—	—	—	—	—	—
Other	—	—	—	—	—	—	—	—	—

TABLE II (*continued*)

Determinator:	Height			Weight			Body Index		
Sub-Group:	Short	Med.	Tall	Light	Med.	Heavy	Low	Med.	High
No. (Boys	13	14	15	11	13	18	10	17	15
of (Girls	24	13	20	19	20	18	18	16	23
TOTAL	37	27	35	30	33	36	28	33	38
LUNGS:									
Expansion									
Good	67.6	70.4	74.3	56.7	75.8	75.0	53.6	69.7	81.6
Poor	32.4	29.6	25.7	43.3	24.2	25.0	46.4	30.3	18.4
Resonance									
N. A. D.	100.0	96.3	100.0	100.0	97.0	100.0	100.0	100.0	97.4
Impaired	—	3.7	—	—	3.0	—	—	—	2.6
Air Intake									
N. A. D.	97.3	100.0	100.0	100.0	97.0	100.0	100.0	100.0	97.4
Impaired	2.7	—	—	—	3.0	—	—	—	2.6
Bronchial Breathing	—	3.7	2.9	—	3.0	2.8	—	10.0	—
Rales	13.5	18.5	17.1	20.0	18.2	11.1	17.9	18.2	10.5
Rhonchi	2.7	7.2	8.6	6.7	3.0	8.3	7.1	3.0	7.9
Other	—	—	—	—	—	—	—	—	—
HEART:									
Apex Beat									
N. A. D.	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Other	—	—	—	—	—	—	—	—	—
Percussion									
N. A. D.	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Other	—	—	—	—	—	—	—	—	—
Rhythm									
N. A. D.	100.0	100.0	100.0	100.0	100.0	97.2	100.0	97.0	100.0
Other	—	—	—	—	—	2.8	—	3.0	—
Murmurs									
Systolic	—	7.2	—	—	6.0	—	—	6.1	—
Diastolic	—	—	—	—	—	—	—	—	—
ABDOMEN:									
Flat	45.9	55.6	48.6	53.3	51.5	44.4	57.1	51.5	42.1
Protuberant	51.4	40.7	42.9	43.3	42.4	50.0	32.1	45.5	55.3
Flaccid	8.1	7.2	8.6	6.7	12.1	8.3	10.7	15.2	2.6
Enlarged Liver	2.7	3.7	2.9	3.3	3.0	—	3.6	—	2.6
Enlarged Spleen	—	—	—	—	—	—	—	—	—
Other	—	—	—	—	—	—	—	—	—
INGUINAL GLANDS:									
N. A. D.	59.5	48.1	62.9	56.7	54.5	58.3	57.1	45.5	65.8
Enlarged	40.5	51.9	37.1	43.3	45.5	41.7	42.9	54.5	34.2
HERNIA:									
Umbilical	—	—	—	—	—	—	—	—	—
Inguinal	—	—	2.9	—	3.0	—	3.6	—	—
GENITALS:									
N. A. D.	84.6	57.1	86.7	90.9	61.5	72.2	90.0	76.5	60.0
Other	15.4	42.9	13.3	9.1	38.5	27.8	10.0	23.5	40.0

light) were also least frequent among them. No significant differences were found in respect of the condition of the children's subskin tissue and musculature.

Of special interest is the fact that no significant differences between the three groups were found in regard to posture.

Of the remaining results of the clinical survey we draw attention to the fact that no significant differences were found in respect of percentage incidence of cheilosis and angular stomatitis, of spongy gums, enlarged and septic tonsils and of enlarged neck glands.

Absenteeism: No statistical difference of relevance was revealed in respect of the rates of absenteeism of the three groups. The lightest group attended school in a slightly more regular manner and although statistically the results are not significant for our sample, the fact that they topped the list in three of the four terms as well as in the total average for the year, suggests the possibility of significance.

Physical Efficiency: The heaviest boys as well as the heaviest girls showed the best physical performances. The differences between the lightest and heaviest performers amounted to 2.1 and 2.5 seconds in the 100 yards race for boys and girls respectively, to 19.9 and 9.5 seconds in the 600 yards race and to 10.1 and 11.1 inches in the shotput. At the age 6-7 these differences are equivalent to a standard growth progress of mean performance values of about 2 years. The above results are significant. Generally heavy body weight, i.e., body weight markedly in excess of the mean for a given group, represents a handicap in the 600 yards race but a distinct advantage in the shotput. From the fact that in our sample the heaviest children should be the best performers in all three tests, we conclude that the entire group of children were considerably under-weight and that their physical performance level as a whole was thus distinctly depressed.

Scholastic Performances: No significant differences were found between the scholastic performances of the three groups.

Social Background: No significant differences were found in respect of the number of persons with whom the children shared their bedroom, as well as to the monthly income average per head of household.

Determinator III: Body Index

Body indices were established for every child in a sample of 114 in accordance with Kaupp's formulae $\frac{w}{h}2$ in which w stands for weight and h for height. The entire range of measurements thus obtained (1.390-1.620) was divided into three equal sectors, boys and girls being grouped separately. The means of the three groups differed significantly.

Height and Weight: There was no significant difference between the three groups in respect of height. The position was opposite, however, regarding weight. The lowest body index groups had mean weights of 41.8 lbs. (boys) and 40.2 lbs. (girls) as against 48.5 lbs. (boys) and 46.1 lbs. (girls) in the highest body index groups. The differences are most significant.

TABLE III

Absence from School
(Figures represent missed days in terms of Percentage of Total Possible School Attendance).

Determinator:	Height			Weight			Body Index		
Sub-Group:	Short	Med.	Tall	Light	Med.	Heavy	Low	Med.	High
No. of Children:	41	32	41	36	37	41	39	33	42
1st Term	10.2	14.7	10.3	13.3	10.3	11.0	13.2	10.8	10.5
2nd Term	13.5	14.6	20.8	18.0	18.0	13.6	15.6	17.4	16.4
3rd Term	5.9	6.2	7.2	8.1	5.8	5.5	7.1	5.4	6.7
4th Term	5.7	7.4	7.2	6.6	6.0	7.7	6.8	5.5	7.8
Total:	9.2	11.2	11.4	11.9	10.2	9.7	11.1	10.0	10.5

Health: 71.4% of the low group as against only 15.8% of the high body index groups belonged to the linear or asthenic type. Conversely, the incidence of lateral or bulky types was 3.6 for the low and 42.1 for the high body index groups. The latter had relatively the healthiest skins. 23.7% of them as against 10% and 10.7% of the two other groups had normal elastic skins. They also showed the smallest incidence of dry (65.8 as against 81.8 and 85.7%) and of rough (28.9% as against 51.5 and 46.4%) skins. None of them had dermatitis and only 5.3% of them as against 18.2% and 21.4% of the other two groups had scabies.

The high body index group were the "best" in regard to their

sub-skin tissue and musculature. In regard to a number of posture features the high body index group was superior, although the incidence of kyphosis and flat feet was approximately the same in all three samples.

No significant differences were found in respect of the condition of the eyes, ears, nose, lips, tongue, teeth, gums, pharynx, neck and inguinal glands of the children. However, there were more cases of flat chests and flaccid abdomens among the low and medium body index groups than were in the high body index.

Absenteeism: No significant differences were found between rates of absenteeism from school.

Physical Efficiency: The boys belonging to the highest body index group topped the list in the 100 and 600 yards races as well as in the shotput test. The extreme differences amounted to 1.5 and 6.9 seconds and to 8 inches respectively. As far as the girls are concerned, we found a significant superiority of the high body index group in the shotput. In the 100 yards race no significant differences were seen, while in the 600 yards race the medium body index group was distinctly in the least.

Scholastic Performances: There were no significant differences between the scholastic performances of the three groups.

Social Background: There were no significant differences in respect of the number of persons with whom the children shared their bedroom as well as of the monthly income average per head of household.

Discussion

In our sample, tallness was a distinct asset in respect of physical efficiency, while shortness was a handicap. This finding corroborates previous statements to the effect that mechanical body features are the most powerful determinators of certain athletic performances. With regard to the other factors tested in this investigation, the shortest boys and girls were not at all at a disadvantage. They had rounder chests, a better musculature and well developed subskin tissue. Also they were slightly more regular in their school attendance.

Weight apparently is a more valid indicator of physique and of nutritional status than is height. The heaviest children were taller and more bulky than the light ones. The fact that among them was found a significantly lower incidence of cutaneous manifestations of

TABLE IV

PHYSICAL EFFICIENCY

Determinator:	100 Yards (in secs.)									600 Yards (in secs.)									Shotput (in lbs.)								
	Height			Weight			Body Index			Height			Weight			Body Index			Height			Weight			Body Index		
Sub-Group:	Short	Med.	Tall	Light	Med.	Heavy	Low	Med.	High	Short	Med.	Tall	Light	Med.	Heavy	Low	Med.	High	Short	Med.	Tall	Light	Med.	Heavy	Low	Med.	High
No. of																											
Boys	12	11	16	10	12	17	11	13	15	12	11	16	10	12	17	11	13	15	12	11	16	10	12	17	11	13	15
Girls	24	13	22	22	18	19	26	12	21	,24	13	22	22	18	19	26	12	21	24	13	22	22	18	19	26	12	21
Means for																											
Boys	20.9	20.2	19.0	21.3	19.8	19.2	21.0	19.6	19.5	185.0	178.4	163.8	188.5	170.8	168.6	178.9	173.4	172.0	44.8	44.5	53.7	43.4	45.3	53.5	44.3	47.3	52.3
Girls	24.2	21.6	21.0	23.7	22.2	21.2	22.5	22.0	22.6	205.4	187.6	178.0	193.0	197.4	183.5	188.1	182.2	200.4	35.6	40.4	45.4	35.3	40.0	46.4	37.8	38.0	44.7

TABLE V

SCHOLASTIC PERFORMANCES EXPRESSED IN PERCENTAGES OF MEAN FOR ENTIRE SAMPLE

Determinator:		Height			Weight			Body Index		
Sub-Group:	Short	Med.	Tall	Light	Med.	Heavy	Low	Med.	High
No. (Boys	12	15	17	10	16	19	11	17	16
of (Girls	26	17	20	22	21	19	25	14	24
Total	38	32	37	32	37	38	36	31	40
Mean: (Boys	97.1	96.1	98.5	99.6	97.5	95.9	100.8	92.9	99.6
(Girls	91.7	92.7	96.2	96.1	89.9	94.4	96.6	95.7	88.8
Total	93.4	94.3	97.2	97.2	93.0	95.1	97.9	94.2	93.1

TABLE VI

MONTHLY INCOME AVERAGE PER HEAD OF MEMBER OF HOUSEHOLD AND NUMBER OF PERSONS WITH WHOM EACH CHILD SHARED ITS BEDROOM

Monthly Income Average Per Head of Member of Household

Determinator:		Height			Weight			Body Index		
Sub-Group:	Short	Med.	Tall	Light	Med.	Heavy	Low	Med.	High
No. of Children:	32	26	30	29	31	28	32	28	28
	£. s. d.	£. s. d.	£. s. d.	£. s. d.	£. s. d.	£. s. d.	£. s. d.	£. s. d.	£. s. d.
Means:	3.14.10.	3.10. 9.	4. 3.10.	4. 0. 2.	3. 8.11.	4. 1. 7.	4. 3. 0.	3.10. 8.	3.15. 4.

Number of Persons with whom each Child Shared its Bedroom

Determinator:		Height			Weight			Body Index		
Sub-Group:	Short	Med.	Tall	Light	Med.	Heavy	Low	Med.	High
No. of Children:	32	26	30	29	31	28	32	28	28
Means:	2.3	1.7	3.0	2.3	2.0	2.8	2.5	2.4	2.3

nutritional deficiencies and of impetigo and scabies than was present among the lighter boys and girls is revealing. The heaviest children were better athletic performers than the light ones. This result invites comment. Generally heavy individuals are handicapped in feats of endurance (e.g., 600 yards running) but excel in performances demanding the display of strength (e.g., putting the shot). Conversely, light weights possess good endurance but little strength (9). The fact that the usual weight-performance correlation was absent in our sample is explained by the assumption that the children were altogether underweight and that therefore only the heaviest among them approximated normal standards.

In respect of body indices, those children who ranked highest were, of course, significantly heavier than the other two groups, and vice versa. However, they were not taller than the others. As one would

expect, asthenic body types were most frequent in the lowest body index group, in which there was also seen the greatest incidence of poor sub-skin tissue and under-developed musculature. The children with the lowest body indices also possessed the least healthy skins (manifestations of nutritional deficiencies and of scabies and impetigo). In the case of the boys, high physical efficiency was correlated with high body indices. However, the correlation was not as high as it was in respect of height and weight. In the case of the girls, the medium body index group produced the best 600 yards runners, while the highest body index group excelled in the shotput test.

For the type of child studied in this investigation, it is obvious that tallness is an asset in athletics only if it is associated with good body bulk. Further, it appears that under the social and economic circumstances prevailing in our sample, girls are more likely to become "too fat" to be good all-round athletes than is the case with boys.

Finally, the fact that no correlations were found between anthropometric measurements on the one side and scholastic performances and social background on the other is noteworthy, more particularly since the latter are known to be inter-related.

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BRAIN, HEART, THYROID, ADRENALS, AND HABITAT

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The concept that habitat may be related to the size of the brain, heart, thyroid, and adrenals of certain animals is the object of this study. Riese (1927) indicated that environmental factors produce changes in the brain of the cetacea. Langworthy (1931) and Jelgersma (1934) accounted for the large brain of certain cetacean animals on the basis of development of large correlation centers and great bodily activity. Jelgersma also related the exceedingly small brain of the manatee to its plant diet and sluggish habits.

In this study 166 subarctic and 57 tropical rodents, carnivores and ungulates, 8 cetacean animals, and 1 manatee were examined.[1] As a result no proof is offered that there is a causal relationship, although the regularity of some of the findings implies that environment may be a causal factor in determining organ size.

METHOD

Comparisons of organs of animals of widely differing body weights were made by application of the power formula, the relative growth equation, $Y = b X^k$.[2] If the constancy of k is established for the size increase of an organ in various classes, it seemed plausible that the comparisons of the organ may be made in terms of b of the equation.

In an earlier study the following k values were observed for a large group of typical vertebrates, including fishes, reptiles, birds, and a variety of mammals: brain 0.541, heart 0.85, thyroid 0.836, and ad-

[1]The total numbers vary from gland to gland.

[2]According to von Hösslein, this equation was first applied to surface area determination by Rameaux in 1838. It was applied to glandular size by Snell in 1891 and by DuBois in 1898. Since then it has come into wide use. See Huxley's *Problems of Relative Growth*.

renal gland 0.867. These values were used in establishing the b values in the accompanying tables. The relative positions of the compared animals are given in terms of b of the equation. For example, if the brain showed a consistently higher b value in one group as compared with another with equal k values, it was assumed that the group with a higher b value had a higher cephalization level. Lumer and Anderson pointed out that b values change depending upon units of measurements employed. A comparison of b values when the same units are employed throughout, however, is not invalidated.

Consider the comparison of b values for the brain of the alligator and chimpanzee. The average b value or cephalization coefficient for three alligators in this earlier study was 0.0177 and for three chimpanzees, 1.280. The k value was similar. This numerical difference in the b values is indicative of the relative difference in cephalization of the compared animals. The same procedure was followed with heart, thyroid, and adrenal glands.

Brain

Brain comparisons were made between tropical and subtropical rodents and their near relatives of subarctic areas. Table 1 shows their comparative cephalization standing as expressed by the coefficient b. Rodents have a low degree of cephalization even in the subarctic

TABLE 1
b Values of Brain

No.	Animal	Mean b Value	S.D.	C.V.	Diff.*	Stand. Error of Diff.
28	Tropical rodents	0.0996±0.002965	0.0157±0.00209	15.75%±2.09		
139	Subarctic rodents	0.1262±0.00360	0.0432±0.00250	34.23%±2.05	0.0266±0.00438	S
15	Tropical carnivores	0.3900±0.0165	0.0622±0.01135	16.00%±2.92		
23	Subarctic carnivores	0.6119±0.0365	0.1750±0.0258	28.54%±4.21	0.2119±0.03938	S
14	Tropical ungulates	0.6500±0.0425	0.1580±0.0300	24.46%±4.43		
4	Subarctic ungulates	0.7040±0.0450	0.090 ±0.0318	30.00%±4.32	0.0540±0.045	NS
8	Cetacea	2.2950±0.1031	0.292 ±0.073	12.72%±3.018		
1	Manatee	0.3169				

*Difference refers to differences between b value in each group. S and NS indicate whether difference is significant or not.

forms, illustrating incidentally, the persistence of the inherited size patterns in spite of environmental modifications. The subarctic group (lemming, arctic hare, microtus, and northern red squirrel) exhibits a brain coefficient 21 per cent higher than that of the tropical ones. Statistically this difference is significant since the actual difference is more than six times its standard error.

Aquatic carnivores, having high cephalization coefficients, are responsible in part for the high value of the subarctic group (weasel, grizzly bear, wolf, polar bear, seal, and walrus) as compared with the wild tropical carnivores (mongoose, genet cat, tiger, lion, hyena, leopard, and serval cat). Even independently of them, however, the values for the subarctic group remain higher.

No other group except the cetacea and the primates show comparable cephalization values. This high degree of cephalization indicates association with their extremely cold habitat that makes constant demands for high metabolism. In an unpublished series of experiments, I observed a close relation between brain size and metabolism. It will be recalled, too, that water dissipates heat 27 times faster than does air of the same temperature, and therefore makes demands for high metabolism. Exclusive of the aquatic forms, the subarctic carnivores have a cephalization value of 0.5251 as compared with 0.3900 for the tropical.

The difference in cephalization coefficients between subarctic ungulates (four caribou) and fifteen even-toed tropical ungulates is not statistically significant, but it may indicate a trend.

The cetacean brain was studied by Röse (1925), Riese (1925-27), Langworthy (1931), Fünfgeld (1927) and Jelgersma (1934). In spite of these studies, no one has successfully accounted for the great brain size of the cetacean porpoise and white whale.

Riese and Fünfgeld considered the cerebral cortex of the porpoise as primitive and differing fundamentally from that of land animals. Röse took issue with them on the number of cortical cell layers, but admitted that the cortex is more primitive than that of man.

Langworthy found cortical gray matter poor in cells, and widely spaced in the porpoise (Tursiops truncatus). He said that the enormous cerebral development is "due to the penetration of large numbers of sensory impulses in this region and the formation of correla-

tion centers." He associated development of cerebellum acoustic tracts and their end stations with aquatic habits.

Jelgersma examined the brains of the porpoise, manatee (sirenia), and those of the aquatic carnivores, the seal and otter. He found the porpoise cortex about half as thick as it is in other mammals, but covering almost twice the area. The increased area is aided by additional convolutions. For the manatee (adult preserved specimen) he recorded a brain weight of 240 Gm.

Dr. George Crile and I captured and dissected a manatee (trichechus) weighing 421 kg. with a fresh brain weight of 351 gm. This finding is in marked contrast to my observations on 6 adult white whales with an average brain weight of 2351 Gm. and an average body weight of 396 kg.

With exception of the sylvian fossa, the brain surface of the sirenia is quite smooth—an observation in agreement with Jelgersma. He associates the low degree of "geistige Entwicklung" with the herbivorous habits of the manatee in contrast with the active habits and higher psychic development of the carnivorous cetacea. Food habits of the sirenia combined with habitat in warm seas, shallow bays, and inlets offer a possible explanation for their small brain size. It must be remembered, however, that they form a distinctive order.

Four male and 2 female white whales (Delphinapterus leucas) taken at Port Churchill, Hudson Bay, 1 porpoise (Phacaena phocaena) taken off Key West, 3 hump back whales (Megaptera nodosa Bonn), 1 sperm whale (Physoter catadon), and 1 fin back whale (Balaenoptera physalus) taken off the northern California coast, were collected for this study. In addition, the brain, thyroid, and adrenal glands of 1 blue whale (Balaenoptera musculus) taken off the Queen Charlotte Islands, were donated.

The brains of these cetacean animals are larger than those of any other group. Measured by the relative growth scale, the degree of cephalization approaches that of the primates. The brains of the 6 white whales showed an average coefficient of 2.218 ± 0.103 as compared to the human brain which has a coefficient of approximately 3.35. The manatee in this study had a cephalization value of 0.3169.

This great brain size is difficult to interpret in light of reduction or loss of appendages and of olfactory functions. Taken with the finding that aquatic carnivores like seal and walrus undergo a similar large

brain development in comparison with their land relatives, leads to the conjecture that cold aquatic environment may be the determining factor in producing these large brains. Evidence based upon comparison of rodents, carnivores, ungulates, and cetacean animals indicates that the animal in the very cold habitat, possesses a higher degree of cephalization than does the animal of similar order in the tropics.

HEART

Heart weight appears dependent upon hereditary and acquired characteristics reflecting the work load of the organ. Rodents show the lowest cardiac coefficients (table 2). While cetacean animals are highly active, they possess the advantage, so far as the work of the heart is concerned, in having their aquatic environment support their bodies. Land animals, on the other hand, must spend large amounts of energy in actual body support. For example, in comparing the organs of the great thoroughbred horse, Equipoise, with those of the white whale of equal body weight, a heart weight of 4455 Gr. was observed in the horse and 3181 Gr. in the white whale. Tropical carnivora can afford different work habits from the northern, since they are not compelled to exert constant activity to maintain body metabolism in face of low external temperatures.

TABLE 2
b VALUES OF HEART

No.	Animal	Mean b Value	S.D.	C.V.	Diff.*	Stand. Error of Diff.
30	Tropical rodents	0.1122∓0.00325	0.0177∓0.00182	15.77%∓2.034		
134	Subarctic rodents	0.1270±0.0064	0.0747±0.0046	5.88%±0.359	0.0148±0.00648	S
20	Tropical carnivores	0.2450±0.0203	0.0908±0.01435	37.06%±0.0585		
20	Subarctic carnivores	0.4350±0.0307	0.1373±0.0217	31.56%±4.98	0.1900±0.0376	S
10	Tropical ungulates	0.3620±0.00580	0.0185±0.00410	5.07%±1.13		
4	Subarctic ungulates	0.5196±0.0580	0.1170±0.04136	22.51%±7.95	0.1576±0.0882	S
7	Cetacea	0.3722±0.0195	0.0519±0.0138	13.94%±3.72		
1	Manatee	0.2059				

*Difference refers to differences between b value in each group. S and NS indicate whether difference is significant or not. Heart B values x10.

Thyroid

Thyroid size is not necessarily an indication of thyroid activity. In exophthalmic goiter, high metabolism may occur without marked enlargement of the gland. Thyroid function in relation to metabolism and its modification with changes in external temperature, is well known. The close relationship between brain size and thyroid size, and more important, the dependence of normal brain activity upon thyroid action, however, has not been so generally studied.

Statistically significant differences appeared when the thyroid coefficients of subarctic and tropical rodents were compared. Differences favored the subarctic group as they did when the thyroid coefficients of tropical and subarctic carnivores were compared (table 3).

The thyroid coefficients of 2 caribou (Rangifer arcitcus arcticus) taken in the northeastern subarctic region of Canada in August and 2 in November were compared with those of 11 African ungulates. Difference in thyroid values, however, was not significant. Fewness of these samples prevent any final conclusions concerning differences.

The following thyroid coefficients of various cetacean animals show

TABLE 3
b Values of Thyroid

No.	Animal	Mean b Value	S.D.	C.V.	Diff.*	Stand. Error of Diff.
20	Tropical rodents	0.0885±0.0165	0.0738±0.0116	83.40%±13.19		
25	Subarctic rodents	0.2056±0.0043	0.0482±0.0034	23.44%±1.41	0.1171±0.01515	S
15	Tropical carnivores	0.4293±0.0744	0.2850±0.0538	66.38%±1.25		
15	Subarctic carnivores	0.6030±0.2600	0.2630±0.0491	43.11%±8.11	0.1737±0.102	NS
11	Tropical ungulates	0.4122±0.0289	0.0960±0.0204	23.28%±4.90		
4	Subarctic ungulates	0.4327±0.0196	0.0392±0.0140	9.05%±3.20	0.0105±0.0296	NS
6	Cetacea (white whale)	2.1020	Fewness of samples precludes accurate estimation or standard deviations and coefficients of variation.			
1	Porpoise	0.3543				
1	Blue whale	0.7649				
1	Manatee	1.1250				

*Difference refers to differences between b value in each group. S and NS indicate whether difference is significant or not. Thyroid and adrenal values are recorded x 1000.

interesting differences: 1 porpoise 0.3543, 2 hump back whales 0.4512, 1 fin back whale 0.4055, 1 sperm whale 0.4464, and 1 blue whale 0.7649.

The high value recorded on table 3 for the 6 white whales is interesting in light of their restricted distribution which limits them entirely to Arctic waters. In comparison with these values the thyroid coefficient of 1.1250 for the manatee again emphasizes the difficulty in evaluating this gland on the basis of size alone.

Adrenal Glands

Histologic studies of the adrenal glands point to a great divergence in the amount of medullary tissue present in different species. In a large alligator the ratio of adrenal medulla to body weight was 1 : 380,000 and in an African lion, 1 : 27,000. Since the gland is apparently not directly concerned with heat production, any correlation between its size and the habitat of its possessor is probably coincidental. Significant differences were observed for the compared animals of this study (table 4).

TABLE 4
b Values of Adrenal Glands

No.	Animal	Mean b Value	S.D.	C.V.	Diff.*	Stand. Error of Diff.
27	Tropical rodents	0.4374±0.0260	0.139±0.0189	31.77%±4.32		
129	Subarctic rodents	0.7240±0.0169	0.192±0.0118	26.51%±1.65	0.2866±0.0293	S
15	Tropical carnivores	0.3883±0.0382	0.150±0.0272	38.63%±7.01		
17	Subarctic carnivores	0.2850±0.0363	0.150±0.0257	53.70%±9.20	0.1035±0.0321	S
11	Tropical ungulates	0.2940±0.0443	0.130±0.0277	40.43%±8.62		
4	Subarctic ungulates	0.5955±0.2000	0.400±0.1410	67.62%±23.71	0.3015±0.0890	NS
6	Cetacea (white whales)	0.7857±0.0382	0.094±0.027	11.9% ±3.46		S
1	Porpoise	0.8989				
Manatee (Adrenal glands not found)						

*Difference refers to differences between b values in each group. S and NS indicate whether difference is significant or not. Thyroid and adrenal values are recorded x 1000.

The adrenal glands in an adult manatee were not located. Dr. W. K. Gregory checked a newborn specimen at the American Museum of Natural History and found a small gland present.

Summary

1. Cephalization measured by the relative growth equation, $Y = b X^k$, was significantly higher in subarctic rodents and carnivores than in tropical ones. It was larger, but not significantly so, in four subarctic caribou compared with tropical ungulates. In the cetacea it was more than three times greater than in any other class examined. Aquatic carnivores showed cephalization coefficients 89 per cent higher than tropical land carnivores. Subarctic land carnivores exhibited coefficients 26 per cent higher than the tropical.

2. Cardiac coefficients were significantly higher in subarctic rodents, carnivores, and ungulates than in their tropical relatives. The heart coefficient of the cetacea was on the small level of tropical ungulates.

3. Higher thyroid coefficients were observed in subarctic rodents and in carnivores than in tropical members of these classes. Six white whales showed thyroid coefficients more than three times those of subarctic carnivores. Ungulates did not show significant intra-order difference.

4. Adrenal glands showed no regular differences correlated with habitat, although subarctic rodents exhibited higher values than tropical ones. Northern carnivores, on the other hand, revealed lower adrenal coefficients than the tropical.

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STUDIES ON THE CELLULAR BASIS OF REGENERATION IN NAIS PARAGUAYENSIS, AND THE EFFECTS OF X-RAYS THEREON

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INTRODUCTION

This paper constitutes publication of a complementary histological portion of a study of regeneration, and the effects of x-rays on the process, in the fragmenting oligochaete, *Nais paraguayensis*.[1] The earlier report, O'Brien, ('42) was limited to: (1) observations on the external features of fragmentation and regeneration; (2) the gross developmental manifestations of x-rayed material; (3) evidences regarding the modus operandi of the x-rays, and the local nature of the regenerative process.

Included in this earlier report are an introduction to the problem and a review of the pertinent literature; for these the reader is referred to the publication to which reference is made above.

The twofold purpose of the present paper is to present and discuss: first, observations on the cellular basis of anterior and posterior regeneration in this oligochaete; and secondly, histological manifestations of x-rayed specimens.[2]

MATERIALS AND METHODS

Nais paraguayensis is a small freshwater oligochaete; it attains a length of 30-40 mm., and measures 0.3-0.4 mm. in width. The body is lightly flesh-colored and quite translucent. It produces asexu-

[1]The author wishes here to express his appreciation of the kindly and helpful criticisms of Prof. S. O. Mast, Dr. Gardiner Lynn and Dr. Chas. Brambel during the course of this work.

[2]The author wishes to acknowledge his indebtedness to Dr. J. E. Wirth and Mr. J. E. Rose, of the Tumor Clinic of the U. S. Marine Hospital in Baltimore, for many kindnesses and facilities extended him, particularly in connection with the x-ray work.

ally by fragmentation; this process consists, essentially, in the spontaneous separation of the animal into several more or less equal parts, without the formation of fission planes generally characteristic of oligochaetes. It is not native to this country. The original culture was obtained through the courtesy of Dr. L. G. Hyman of the American Museum of Natural History, New York. In Hyman ('38) there is included directions for culturing this oligochaete.

For further details and data regarding the methods employed in handling the organism and the conditions under which the x-radiation work was carried out, the reader is referred to the earlier publication, O'Brien ('42).[3]

I. The Cellular Basis of Regeneration

Observations

The following observations on normal regeneration were made by way of analyzing the regenerative processes in *Nais paraguayensis* in terms of the qualities and various activities of those cellular elements entering immediately into the process. Natural and cut fragments were fixed at various times after cutting: immediately, at one, six and ten hours, and at six-hour intervals from twelve to thirty-six hours, and at two, three, four, five and seven days. Cut fragments were used principally, but whole worms also were fixed. The material was sectioned longitudinally and, in some few cases, transversely.

Anterior and posterior healing of cut and natural fragments:

The process of wound closure, both anteriorly and posteriorly, is consummated almost immediately. Internally, the picture of this wound edge bears out the suggestion of its external features, that is to say, there is a fusion of the epidermal and muscle cells of the cut edge. The cut closes by the fusion of the epithelial cells of the body wall; the closed end of the gut is not in immediate contact with the epithelial cells of cap closing the coelom.

This situation obtains at either end of a cut piece. An occasional

[3]The monograph of Stephenson ('30) contains a comprehensive list of references on the gross features of regeneration in oligochaetes. Krecker ('10) and the excellent review of Stolte ('36) include numerous references on the problem of the cellular basis of regeneration in oligochaetes. In Curtis ('36) will be found an extensive list of references on the effects of X-radiation on regeneration. The larger works of Abeloos ('32) and Korschelt ('31) provide excellent references in the problem of regeneration.

mitotic figure was observed in the epithelial layers, while still in the healing stage, but it is nevertheless clear that the closure is essentially a fusion phenomenon. No cicatrix of injured muscle cells at the closed ends was observed, such as is described in *Tubifex* by Stone ('32). No difference was observed in the method of wound closure at different levels of the body.

A few specimens were fixed just previous to and immediately following the separation of fragments at the loci of separation; the histological features revealed in these naturally severed extremities is identical with that in the healing stump of a cut fragment. Further discussion of the cellular picture at these loci will be presented in another connection.

A. Anterior Regeneration

(a) *Initial Proliferative Activity*: Considerable proliferative activity of the ectodermal epithelium in the immediate vicinity of the wound was observed six to ten hours after cutting. Tail pieces were used here because at this early stage it is difficult to distinguish anterior and posterior extremities of trunk fragments regenerating at both ends. This proliferation proceeds rapidly and soon the space between the coelomic cap and the blind end of the gut is filled with cells. The preponderance of cells appears to come from the epithelium of the cap closing the coelom and particularly from that in the vicinity of the severed ventral nerve cord. At about eighteen hours this mass of ectodermal cells appears as an elongated mass of cells extending from the ectodermal cap posteriorly to the old intestine. This thick mass of cells appears to be composed exclusively of ectodermal cells. The epithelium of the old gut undergoes some proliferation anteriorly, but it appears to be a minor factor in the establishment of the connection between the epithelium of the wound surface and the old gut. These initial phases are similar to those described for species of *Nais, Stylaria,* and *Pristina* (Naididae) by Hepke ('97). At about twenty-four hours certain definite segregations of these cells have appeared. The differentiation of various structures of the head will be considered separately.

(b) *The Epidermis and Prostomium:* The new epidermis is formed by proliferation within the single-celled layer of old surface epithelium. The prostomium is apparently not highly specialized and is com-

prised merely of the terminal cap of tissue in association with some of the anterior-most newly proliferated epidermal cells. (Fig. 2, 8)

(c) *Oral Segment and Stomodaeum:* The first definite indication of the differentiation of the oral segment (Segment I) is the formation of the stomodaeal invagination, at about eighteen to twenty-four hours. This latter structure is derived as follows: some cells of the new ectodermal mass grow ventrally toward the ventral body wall; meanwhile there has appeared in the outermost ectoderm an invagination, the stomodaeum; between this invagination, and the cells growing down from the inner ectodermal mass a connection is established (Fig. 8).

(d) *The Pharynx:* The bulk of the pharynx, which occupies a great part of the head (Fig. 2), is derived from the cells of the original thick mass of ectoderm. Its posterior connection with the old gut appears to be established by a fusion of the posterior-most new ectodermal cells and the definitely limited new growth of the old endodermal epithelium, to which reference has been made. The ventral growth of ectoderm cells toward the stomodaeum is really the anterior-most portion of the new pharynx; by the second day a lumen has been established between the mouth and pharynx. Similarly, the continuity of the posterior end of the pharynx and the old intestine has been established (Fig. 2). The region of the old gut immediately behind this newly formed pharynx eventually undergoes a regulation to form the typical oesophagus. These observations on the formation of the stomodaeum and pharynx agree with the accounts given by Hepke ('97) in other species of Naididae.

(e) *Nervous System:* The cerebral ganglia (Fig. 1, 2, 8) are formed by the lateral and dorsal proliferation of epidermal cells of the terminal wound region. As already noted, the epidermis in the immediate vicinity of the severed nerve cord is particularly active. At twenty-four hours the ganglia are well segregated from the remainder of the ectodermal mass, and lie dorsal and slightly anterior to the stomodaeum (Fig. 8). The ganglia subsequently elongate and two conspicuous connectives are given off posteriorly (Fig. 1); these two elongations are the anterior extremities of the posteriorly fused ventral nerve cord. It could not be ascertained precisely how the connection between the new cords and the old cord is established, but it appears to be by a ventral deflection of the former in the immediate

vicinity of the pharynx. In the region of the pharynx numerous small nerves extend peripherally from these trunks and connect with the surrounding body wall (Fig. 2). No part of the new nervous tissue was observed to come from the old cord itself. The origin of the nervous system in the regeneration of oligochaetes has been consistently described as ectodermal by numerous investigators.

(f) *Ventral setal follicles and setal muscles:* On either side of the nerve cord there are formed small aggregations of ectodermal cells from which the ventral setal bundles eventually arise. The muscular complexes associated with these bundles appear to be derived entirely from this same mass of ectodermal cells, although, as will be seen, posteriorly these muscular elements arise from the mesodermal neoblasts.

Little can be said here of the origin of the new longitudinal and circular muscular elements of the head. It would seem that for a proper study of these, special staining methods would have to be used. It seems rather likely, however, that their restoration is brought about by the combined activities of the new ectoderm and old muscular tissue. While there is a prominent complex of blood vessels in the newly regenerated head, it is impossible at present to describe its

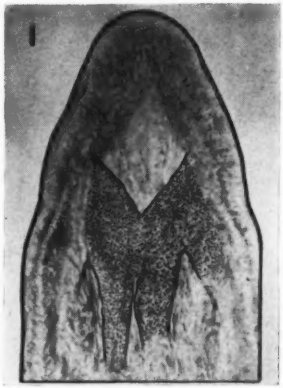

FIGURE 1

Photomicrograph (retouched) of the cephalic extremity of a living adult specimen of *Nais paraguayensis,* showing, in dorsal aspect, the cerebral ganglia and posteriorly directed nerve trunks.

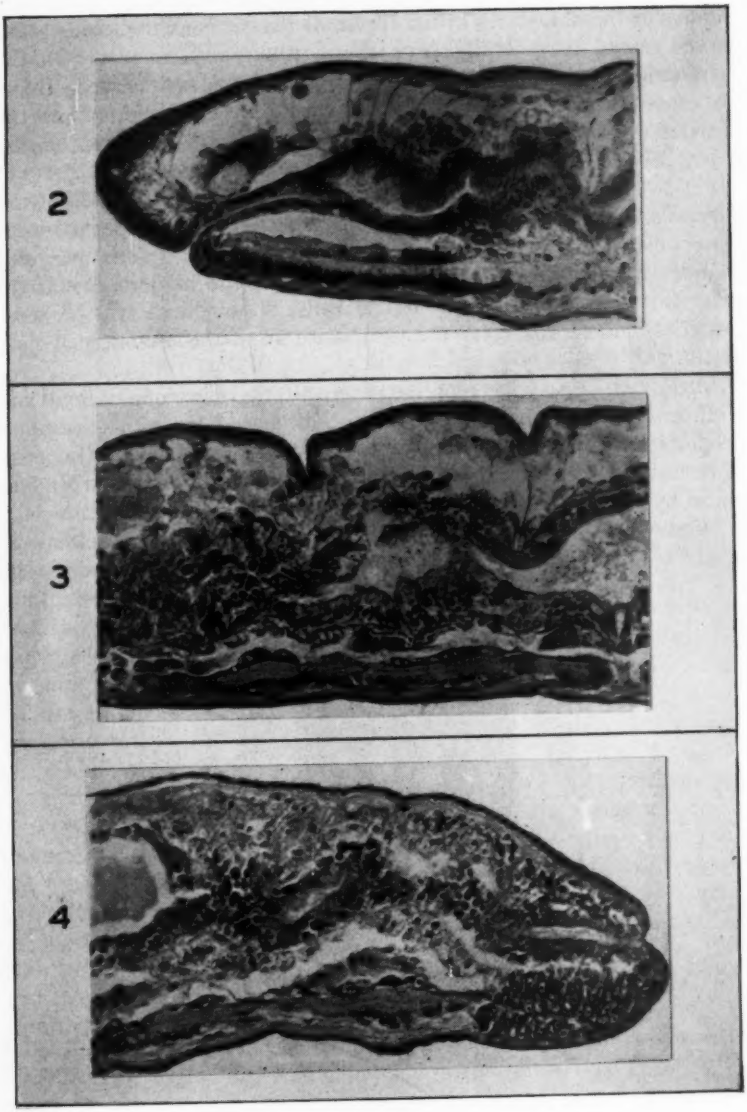
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PLATE I

FIGURE 2

Sagittal section through a newly regenerated head, showing: prostomium, mouth, pharynx, cerebral ganglion, posterior nerve trunk, nerves to body wall, epidermis, ventral blood vessel and ventral nerve cord.

FIGURE 3

Sagittal section through the posterior third of an adult specimen, showing: epidermis, muscle layers, ventral nerve cord, neoblasts, septum, chlorogogue cells, body-fluid cells, ganglion cells and gut.

FIGURE 4

Sagittal section through a posterior regenerate of twenty-four hours, showing: neoblasts on ventral nerve cord, proliferating neoblasts (more posteriorly), ectoderm, regenerating gut, proctodeum and coelom.

(*All figures are photomicrographs of sectioned and stained material*)

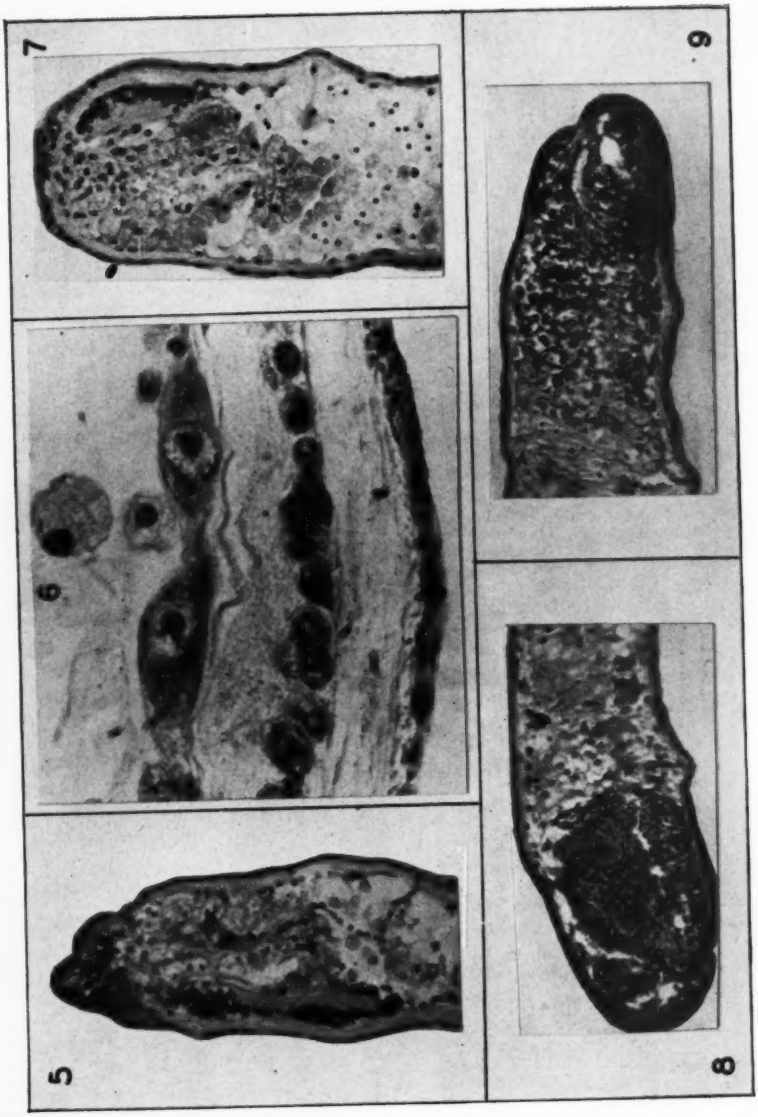
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PLATE II

FIGURE 5

Sagittal section through the anterior extremity of an irradiated fragment (9000 r) showing: the terminal knob of ectodermal cells; no true regenerative activity.

FIGURE 6

Sagittal section through the nerve cord in the mid-body region of an adult specimen, showing, particularly, the large spindle-shaped neoblasts, with large nucleus and nucleolus, lying on the ventral nerve cord.

FIGURE 7

Sagittal section through the posterior extremity of an irradiated fragment (9000 r), showing lack of regenerative activity. An "inactive" neoblast is shown terminally.

FIGURE 8

Sagittal section through an anterior regenerate (non-irradiated) of approximately twenty-four hours, showing: cerebral ganglion, ectodermal cells of new pharynx joining the stomodeal invagination, prostomium, proliferating ventral ectoderm, and old epidermis.

FIGURE 9

Sagittal section through a posterior regenerate (non-irradiated) of approximately twenty-four hours, showing: proliferation of ectoderm and neoblasts, proctodeum.

(*All figures are photomicrographs of sectioned and stained material*)

origin; the staining methods employed did not serve to distinguish these elements too well.

B. Posterior Regeneration

(a) *Initial Proliferation:* Eight to twelve hours after cutting, considerable proliferative activity was observed at the posterior cut extremity. Two kinds of cells are involved in this increase in cell mass: (1) surface epithelial cells and (2) neoblasts (mesodermal). The small compact mass of ectodermal cells quickly establishes contact with the old gut. Meanwhile, neoblasts have appeared at the site of the wound; these cells have "migrated" posteriorly along the ventral nerve cord to the region of epithelial proliferation, whereupon they too begin rapid proliferation, in a position anterior to the terminal ectodermal cap.

A few special features of the neoblasts may be described here. They eventually furnish the material for the regeneration of mesodermal structures posteriorly. They are characteristically large spindle-shaped cells, with a large nucleus and a prominent nucleolus; their cytoplasm is densely granulated (Fig. 3, 4, 6). They occur in the coelom, lying on both left and right portions of the nerve cord, along the entire length of the animal, with the exception of the head region. Their posterior "migration", spoken of previously, is indicated by the fact that they are present in the wound region after cutting in far greater numbers than elsewhere at anytime along the animal (excepting the terminal growing region), and they exhibit certain shapes along the nerve cord, especially near the site of the wound, which are highly suggestive of their active migration. There is some experimental evidence for this migration (cf. O'Brien ('42)).

(b) *Anal Segment and Proctodaeum:* Before the end of the first day after cutting there is a conspicuous proctodaeal invagination in the initial posterior ectodermal growth; the new ectoderm establishes connection with the old gut. The anal opening, although terminal, is slightly dorsal to the median line (Fig. 9); this is in contrast to the stomodaeum which is ventral. This process of anal segment formation is the same at all levels of the body. Ectodermal proliferation continues indefinitely from immediately in front of the anal segment.

(c) *Posterior Gut:* The new gut is replaced by the continued pro-

liferation of ectodermal cells immediately anterior to the anus (Fig. 4). Some proliferation of the epithelium (endodermal) of the old gut apparently occurs, but this seems insignificant in comparison with the ectodermal activity.

(d) *Ventral Nerve Cord:* The new portion of the nerve cord is progressively formed by the proliferation of the ectoderm immediately ventral to the cut end of the old cord. No proliferation from the old nervous tissue was observed.

(e) *Septa:* The neoblasts which have "migrated" to the regenerating tip undergo extensive cell division. As growth in length proceeds the smaller newly formed neoblasts collect in segmentally arranged masses or columns, on either side of the nerve cord. The cells of these closely packed columns extend dorsally and laterally; the columns thus become narrower, and are soon recognizable as the new septa (Fig. 4); the oldest of these newly formed septa is farthest anteriorly from the closely packed cells of the tip.

(f) *The Nephridia:* The nephridia differentiate from ventrolateral aggregations of neoblasts. Turner ('34) has described their regeneration in *Lumbriculus* from large neoblastic "mother cells" which divide repeatedly to form the anteseptal and postseptal portions of each. Detailed processes such as this could not be observed in the present material, but since the structure of the kidney is similar to that of *Lumbriculus,* the process is very likely much the same in *Nais paraguayensis.*

(g) *Blood Vessels:* As was mentioned in connection with anterior regeneration, the restoration of the vascular system could not be clearly ascertained, but some observations indicate that several vessels are formed from originally solid rods of small neoblasts, in which a lumen is subsequently established. Such a picture is in agreement with the reports of other workers, e.g., Randolph ('92), and Krecker ('10).

(h) *Circular and Longitudinal Muscles:* It was difficult to ascertain whether the cells which take up a position adjacent to the epidermis, and which eventually differentiate into the inner circular muscles, are neoblastic or ectodermal in origin; however, their origin from the former stock seems more probable. No indication of the formation of new muscle from old muscle tissue was observed. The situation is similarly unclear as regards the longitudinal muscles.

Perhaps the regeneration of these elements is shared both by ectodermal cells and neoblasts.

(i) *Ventral Setal Follicles and Muscles:* Laterally, on either side of the nerve cord, there are formed small segmentally arranged masses of ectodermal cells, derived from the ventral epithelium; these are the ventral setal follicles which eventually produce the ventral setal bundles. Their origin is similar anteriorly and posteriorly. However, posteriorly, these follicles are surrounded by neoblasts which differentiate into a muscular complex associated with each bundle. Anteriorly, these muscles appear to be of ectodermal origin.

(j) *The Neoblasts:* The salient cytological features of the neoblasts have already been described. Regarding their distribution, they occur in the ventral coelomic cavity, on either side of the nerve cord (Fig. 3, 4, 6). They were not found in the head region proper, and are less numerous in the anterior-most four to six millimeters of the trunk region than in its remainder. They are present in uninjured adult worms as a persistent cell stock, and no indication was found of their development from peritoneal cells or "quiescent neoblasts" on the septa, such as has been described by Stone ('32) in *Tubifex* and Krecker ('23) in *Tubifex* and *Limnodrilus*. They migrate only posteriorly and were not found to participate in the regeneration of the head. The histological evidence for their migration has already been discussed. There does not appear to be any orderly differential distribution of these cells along the antero-posterior axis of the adult worm.

Discussion

In the beginning it was thought that there might possibly be some differential distribution of the neoblasts correlated with the loci of fragmentation appearing in adult worms, that is to say, that they might be found to be more numerous at these sites, as they are in the fission or proliferation zone in *Dero,* Stolte ('38). However, no such distribution of these cells was observed; their accumulation at the posterior extremity of fragments takes place only some hours after the separation is completed. As a matter of fact, there appears to be little rhyme or reason in the antero-posterior distribution of neoblasts in adult worms. It is noteworthy, however, that they are absent in the immediate head region and are scarcer in the first three

to five millimeters of the trunk than in other trunk regions; and it is approximately this region, which corresponds to the naturally produced head fragment which is always longer than the remaining fragments (cf. Hyman ('38) and O'Brien ('42)).

It also seemed conceivable that since the neoblasts are so important in posterior regeneration, a limited anterior distribution of these cells might be causally associated with the fact that a certain number of trunk segments (three) must be left attached to a head in order that posterior regeneration can occur; however, neoblasts were observed anterior to the second trunk segment (the one from which posterior regeneration will not occur). Nor can this regenerative disability spoken of be explained, as has been suggested, by the absence of a nephridium in these trunk segments, for in *Nais paraguayensis* a nephridium occurs in segment VII, i.e. the second trunk segment. The observations on the regeneration of two-segment pieces, taken from regions of the body where neoblasts are generally numerous, suggest that it is some physiological "dominance effect" of the growing anterior extremity which inhibits the formation of a tail, rather than the absence of any cellular elements necessary to that end. Thus it must be said that these observations support the "physiological explanation" according to which this failure of posterior regeneration is the result of a physiological "dominance" exerted by the anterior region. (cf. Hyman ('38))

Stone ('33) in discussing the inability of *Tubifex* to regenerate anteriorly if more than twelve anterior segments are removed, and noting the failure of neoblasts to appear and migrate anteriorly during normal regeneration in this form, suggests that the limited anterior region in *Tubifex* within which regeneration (anterior) occurs may be correlated with the absence of neoblasts; genera like *Lumbriculus* and *Aelosoma,* in which neoblasts do migrate anteriorly, regenerate a head from almost any level of the body. On the basis of this suggestion, the ability of *Nais paraguayensis* to regenerate anteriorly from any level of the body (except the extreme posterior end) might be explained; for although the neoblasts do not migrate anteriorly (from quiescent neoblasts on the septa) they are already present in their definitive form throughout the length of the animal, except in the head region proper. However, in this connection it must be remembered that, in the present account, neoblasts were

not found to enter into anterior regeneration; it is not unlikely that they do so in connection with the restoration of muscular and vascular elements, the origin of which, as has been stated, has not yet been determined.

The process by which the ectoderm enters upon its regenerative activity apparently involves first a dedifferentiation followed by a redifferentiation. The neoblasts on the other hand appear as reserve undifferentiated material; that the neoblasts are less differentiated than the ectoderm will be shown experimentally in the following section.

It is conceivable that the greater speed with which anterior regeneration is consummated at the outset of the process, may be correlated with the fact that it does not have to wait upon the arrival of neoblasts; whereas the initial ectodermal phase of posterior regeneration will not proceed further until neoblasts have migrated to the vicinity and have begun to proliferate.

Stolte ('35) has proposed that on the basis of their habitat, the oligochaetes fall into three groups, which are distinguished by the character of their blastocytes (regenerative cells): (1) *the inhabitants of free-water* in which the blastocytes as neoblasts occur along the entire body, and may migrate from the coelom into the various tissues; (2) *the inhabitants of mud* that have the blastocytes present as basal cells from which neoblasts develop into the regenerative processes; (3) *the more definitely terrestrial group* in which blastocytes are present as basal cells or as neoblasts which arise by dedifferentiation of muscle cells. Clearly, *Nais paraguayensis* is a typical member of group (1); the present study however revealed no migration of the neoblasts into various tissues such as Stolte ('33) has described in *Dero limosa*.

Histology of Irradiated Specimens

Observations

In the light of the generally established fact that x-radiation effects curtailment of regeneration by way of a deleterious effect upon the regenerative cells, it was quite natural to look first to any changes occurring in those cellular elements already found to be responsible for the restoration of parts in the normal regeneration of this oligo-

chaete, that is to say, principally the ectodermal epithelium and neoblasts, and, to some extent, the endodermal epithelium.

A—*Anterior Inhibition:* As already indicated, the process of wound healing in irradiated specimens appears externally to be identical with that in normal specimens. Histologically, the closure of the body cavity and gut of irradiated worms was found to be no different from that already described for normal specimens. However, in the irradiated specimens there follows no proliferation of the ectodermal epithelial cells in the vicinity of the cut, such as is characteristic of the normal regenerative process. There is generally a thickening of the cap of cells of the stump, but this is primarily the result of a general random crowding and rearrangement of the tissue of the cap. While there is apparently some proliferation of ectodermal cells in this cephalic knob, it appears to be a minor factor in its formation. Enlarged, abnormally appearing ectodermal cells are found in this somewhat thickened mass. Figure 5 shows the internal features typical of a rather completely inhibited anterior extremity. The nuclei of the cells of this irregular mass or cephalic knob stain rather heavily, but only occasionally was a mitotic figure observed. The epithelium and muscle layers often appear less compactly joined in the vicinity of the wound. The intestine of irradiated specimens likewise closes to form a blind tube as it does in normal worms, but there is not established any substantial connection between the two epithelia as occurs normally. No mitotic figures were observed in the irradiated endodermal epithelium. The smallest cephalic knobs appear to be formed by the irregular folding of the tissue comprising the epidermal cap. However, in larger knobs some proliferation of the ectoderm occurs, but it is slight at best. Abnormally large and small cells are often found in this mass. The indication of a stomodeum appears to be nothing more than a localized indentation in this irregular ectodermal mass. In these knobs, numerous loose muscle cells are often intermixed with the ectodermal cells. This situation just described obtains in irradiated specimens a week or more after exposure.

Clearly the radiation has so affected the ectodermal epithelium as to render it incapable of proliferation. Hence is precluded the formation of those structures which, as described previously, are regenerated through the growth and differentiation of ectodermal elements, and to some slight extent by the epithelium of the gut.

B—*Posterior Inhibition:* The process of healing posteriorly is the same externally and internally in irradiated specimens as it is in normal specimens. It will be recalled that the formation of the tail is dependent upon the activities of the ectoderm and the neoblasts, and it is to the inhibitive effect of the rays on these elements that the inhibition of posterior regeneration is due. Regarding ectodermal activity in tail formation, which begins with the formation of the anal segment, this is inhibited less completely with a given dose than is the activity of the neoblasts; that is to say, an apparently normal anal segment can be formed, although neoblast activity, as expressed by migration to the site of the wound and subsequent proliferation, is definitely checked; the anal segment remains as is, and no activity of the neoblasts is observed. The formation of the smallest anal knobs (not typical anal segments) appears to proceed in the manner described for the cephalic knobs; that is, it is essentially a rearrangement of the cap of tissue closing the coelom, rather than regeneration. The indication of an anus in these knobs likewise appears to be no more than a mere indentation in this mass of cells. The relatively few cells of the knob are principally ectodermal and not closely packed. Occasionally a few neoblasts are present in the immediate vicinity of the knob, but these generally appear abnormal and are definitely inactive. When stronger doses are applied so as to preclude the formation of even a knob, there still may be a few inactive neoblasts situated very close to the inner surface of the cap of tissue closing the coelom (Fig. 7).

Normally, as already seen, the activity (proliferative) of the neoblasts begins just anterior to the anal segment; but the radiation so affects them as to prevent both their migration posteriorly and their proliferation. The effect is not merely one of curtailing their activities, but it also occasions their eventual disintegration or degeneration; indications of this degeneration were detected in some specimens fixed within three-quarters of an hour after exposure, and although others survive degeneration considerably longer, they do not become active. The necrotic condition found in the irradiated neoblasts, as in the ectoderm, is not easy to characterize; that is to say, the abnormalities appear to be such as one might expect from the application of most any lethal agent. The most conspicuous abnormalities in many such neoblasts are a pronounced reduction in the

cytoplasmic content, an irregular contour of the nuclear wall, and a condensed condition of the nucleolus. But even after a day or two, several apparently normal neoblasts can be found in the ventral coelom, but these betray no activity, and are relatively very few as compared with the number in non-irradiated controls. Most generally the nerve cord of irradiated specimens is quite denuded of neoblasts after a day or two, and disintegrating neoblasts appear as conspicuous dark masses along the cord and in the posterior terminal region.

Thus ectodermal inhibition together with the inhibition and ultimate destruction of the neoblasts eliminates the possibility of the restoration of those organs for which these cellular elements are responsible in normal regeneration.

Discussion

From the observations on the normal regenerative process in *N. paraguayensis*, it appears that the role of the ectodermal epithelium in regeneration is accomplished first by way of a *dedifferentiation* and then a subsequent *redifferentiation;* such a process of differentiation of ectoderm has also been described by Hammerling ('24) in *Aelosoma,* and by Stolte ('33) in *Dero.* (Schotte ('40) has rather conclusively proved the occurrence of a true dedifferentiation and a subsequent redifferentiation in the formation of the regeneration blastema in urodeles). On the other hand, the neoblasts of *N. paraguayensis* (and other naids) appear to be a stock of definitely undifferentiated cells, set aside in the adult worm as a "reserve" for regeneration. Thus, on the basis of the "law" of Bergonie and Thribondeau ('06), which states that the sensitivity of cells to radiation varies directly with their reproductive capacity and inversely with their degree of differentiation, it is reasonable to expect that the ectoderm would be less inhibited (in its regenerative activity) by a given dose of radiation than would the less differentiated neoblasts. And, as a matter of fact, as has been seen, this is true, both as regards the predominantly ectodermal activity of anterior regeneration and the initial ectodermal phase of posterior regeneration. Thus these observations provide some experimental evidence regarding the relative degree of differentiation of the regenerative elements in *N. paraguayensis*, and lend further support to the above generalization concerning the differential susceptibility of cells to radiation.

It will be recalled that the initial ectodermal phase of posterior regeneration can proceed in some irradiated specimens in an apparently normal manner, but then ceases; this cessation is associated with the definite inhibition of neoblastic activity. It is conceivable that if the activity of the neoblasts could be renewed in such specimens, the ectodermal activity might as a consequence, be resumed. Such a situation would assign a "stimulatory" role to the neoblasts. Certain experimental work on *N. paraguayensis*, reported in O'Brien ('42), as well as some work of Zhinkin ('34) on *Rynchelmis* suggest that such a role of the neoblasts is not unlikely.

It is difficult to say here whether the destructive effect of the radiation on the ectoderm and neoblasts is to be thought of as occurring principally in the nucleus or cytoplasm. The present methods were not suited to the study of this question. It would be most fortunate if these cells could be grown *in vitro* and radiation applied in varying doses; in this way the progressive effects of the radiation upon the various elements of the cells could be more profitably studied.

The fact that the typical post-cephalic regulation of the intestine does not take place in specimens in which the head has been completely (or almost completely) inhibited presents an interesting problem, which however falls outside the present scope. It may be mentioned, however, that this observation on *N. paraguayensis* is in a manner similar to that of Brønsted ('39) who demonstrated by a new transplantation technique that in regeneration in planarians, the induction of new organs becomes impossible under conditions in which no blastema is allowed to form.

Summary

Anterior regeneration: (1) The major organs of the newly regenerated head are of ectodermal origin. (2) The origin of the muscles of the body wall and the cephalic blood vessels was not ascertained. (3) The participation of neoblasts in anterior regeneration was not observed. (4) The following structures were found to be of ectodermal origin: New ectoderm, prostomium, stomodaeum, pharynx, cerebral ganglia and their extensions, ventral setal follicles, and the setal muscles. (5) The head as a unit may be looked upon as predominantly ectodermal.

Posterior Regeneration: (1) Neoblasts, a special type of regener-

ative cell, constitute a persistent cell stock in the adult worm. (2) Subsequent to cutting, neoblasts migrate to the site of the wound where they undergo proliferation and enter actively in association with the ectoderm into the regenerative process. (3) The anal segment (with proctodaeum), gut, nerve cord and setal follicles are ectodermal in origin. (4) The septa, nephridia, ventral setal muscles, and (most probably) muscles of the body wall are derived from cells provided by the proliferation of the neoblasts.

Effects of x-radiation: (1) X-radiation curtails the regenerative process by way of a selective inhibitive effect upon those cellular elements, through the activity of which normal regeneration is accomplished. (2) The mesodermal neoblasts, particularly active in the regeneration of the tail, are more susceptible to irradiation than the ectodermal epithelium, the activity of which predominates in anterior regeneration. (3) Radiation not only inhibits the migratory and proliferative activity of the neoblasts but also causes their eventual disintegration. (4) The small knobs formed anteriorly and posteriorly in irradiated specimens, and often bearing stomodaeal and proctodaeal invaginations, do not represent true regenerative activity.

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EFFECTS OF THE GROWTH INHIBITOR, HEXENOLACTONE, ON FROG EMBRYOS. I. EFFECTS ON DIPLOID EMBRYOS. II. DIFFERENTIAL EFFECTS ON HAPLOID AND DIPLOID EMBRYOS

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According to work by Medawar et al ('43) and Kuhn et al ('43) δ-hexenolactone is the active chemical in growth inhibiting extracts of malted grains, orange peels, Sorbus berries, and certain other natural products. Special interest is attached to hexenolactone because it, like the extracts, is supposed to inhibit the growth of mesenchymal cells more than that of epithelial ones. The original purpose of testing the effects of this chemical on frog embryos was to determine if it would also suppress specifically the *differentiation* of mesenchyme or other mesodermal structures.

In the course of the first few experiments it was found that hexenolactone was very effective in stopping early cleavage. This result suggested that the chemical might have important cytological effects, and in this connection we were particularly interested to know if changes in chromosome number could be produced by it. If so, hexenolactone would become very useful as a general tool in animal embryology and cytogenetics, and would also provide us with a means of approaching one particular problem in amphibian embryology—namely, the problem of the interpretation of the haploid syndrome of morphological deficiencies and abnormalities (see review by Fankhauser, '45).

With these points in mind we made comparative studies of the effects of hexenolactone on haploids and diploids. We found that there were no special inhibitory effects on the differentiation of mesenchyme or other mesodermal derivatives as a class, and that changes

[1]I should like to acknowledge the valuable assistance of Miss Lily Sakaguchi who did much of the microtechnical work in this investigation.

in chromosome number could not be brought about. However, other striking effects were produced in the embryos, and haploids and diploids proved to be distinctly different in sensitivity to the lactone. These results and the implications that they have regarding the site of action of hexenolactone in the cell at different stages of development are presented in what follows.

Materials and Methods

The frogs used in these experiments were *Rana pipiens*, all obtained from the northern Lake Champlain region of Vermont during Winter and Spring, 1943 to 1945. Eggs were obtained by the usual method of pituitary injection. Experimental embryos were reared in 10% Ringer's solution made up with glass distilled water. Stock embryos were kept in tap water that was thoroughly aërated before the embryos were placed in it. The experiments were run throughout at a temperature of 18°-19.5°C.

Delta hexenolactone, m.w. 112, b.p. 103-105°C (14mm), was synthesized by Dr. A. P. Swain of the McNeil Laboratories, Philadelphia, according to the method of Kuhn and Jerchel ('43). I am indebted to Dr. Swain for giving me a liberal supply of the chemical. The pure hexenolactone, a clear, colorless, rather viscous liquid, was stored at 4°C in sealed ampules under nitrogen. At the beginning of each experiment a stock solution was made up in distilled water in the amount of 100 mg of hexenolactone in 100 ml of solution (100 mg%). This was then diluted with 10% Ringer's solution to give test solutions usually ranging in concentration from 0.01 mg% to 5 mg%. The test solutions were usually changed daily although this appeared not to be strictly necessary inasmuch as the biological effects were the same when the solutions remained unchanged for periods of 3 or 4 days. Stock solutions, kept at 4°C, were generally used for one week only although in this case too solutions a month or more old seemed just as effective as freshly made up ones. The pH of the hexenolactone solutions was always the same as that of the diluted Ringer's—about 7.2.

Experimental embryos were reared in finger bowls, about 25 in 90 ml of solution; or in Stender dishes, about 10 in 30 ml of solution. In some experiments the jelly was removed from the eggs; in others

jelly plus vitelline membrane were removed from late gastrula embryos. The effects of the chemical on development were exactly the same whether these structures were present or not. Fixations were usually done in Smith's ('15) fluid and occasionally in Helly's fixative. The embryos were run up through amyl acetate to tissuemat (Drury, '41), sectioned at 10μ, and stained with the Feulgen reagent and fast green.

Androgenetic haploids were produced by removing the second polar body spindle from the egg about 20 to 40 minutes after insemination, according to Porter's ('39) method. This method is an excellent one, and with a little practice it was possible to make as many as 80 haploids from one clutch of eggs inseminated in two groups about 20 minutes apart. The removal of the second polar body spindle involved the formation of a small exovate containing the female nucleus plus a little of the egg cytoplasm. In some experiments exovates containing only cytoplasm were made on the diploid controls. These eggs developed perfectly normally and reacted to hexenolactone just as did the untreated diploids.

I Effects of Hexenolactone on Diploid Embryos

To begin with we made a general survey of the effects of hexenolactone on the development of diploid embryos in order to provide a basis for the comparison between haploids and diploids.

1. External morphology: The effects of the lactone on cleavage are summarized in table 1. It will be seen that concentrations of 1 mg% or lower had no influence on the rate of appearance of the first three cleavages. In 5 mg% the eggs cleaved once or twice over a period of two hours and then stopped; in 10 mg% only one cleavage occurred at a definitely reduced rate during the first hour of exposure and there was no development beyond that time. Eggs placed in 100 mg% hexenolactone stopped developing and began to cytolyse within the first half hour of exposure.

The post-cleavage development of eggs exposed continuously to the chemical is illustrated in figure 1. The concentrations ranged from 5.0 mg% to 0.01 mg%; exposure was begun shortly after insemination (St. 2 or 3 of Shumway, '40); at the beginning of gastrulation (St. 10); at the end of gastrulation (St. 12); and in late tail bud stage (St. 18—). Examination of figure 1 shows that there was a

TABLE 1

EFFECTS OF HEXENOLACTONE ON THE FIRST THREE CLEAVAGES OF RANA PIPIENS EGGS
Eggs placed in test solutions either 1 hour after insemination or at time of appearance of first cleavage furrow at 2½ hours after insemination.

Concentration of hexenolactone mg./100 ml.	Number of eggs	Result
0.0 (cont.)	80	Normal cleavage
0.01	50	Normal cleavage
0.1	50	Normal cleavage
1.0	80	Normal cleavage
5.0	55	Cleave once at retarded rate (ca. 5 min. behind controls). May cleave abortively a second time. Stop developing within 2 hours.
10.0	25	Cleave only once—about 9 minutes behind controls. Stop developing within 1 hour.
100.0	25	No cleavage. Eggs begin to cytolyse within 30 minutes.

definite decrease in sensitivity to the chemical as the embryos developed. Thus while eggs placed in 5.0 mg% hexenolactone shortly after fertilization developed for only 2 hours and cleaved only once or twice, those placed in the same concentration at the beginning of gastrulation developed for about 8 hours during which time the dorsal lip progressed to the crescentic form; and those placed in this solution at the end of gastrulation developed for 12 to 16 hours and formed the medullary plate and folds. In the latter embryos the medullary tissue, once formed, was the first to disintegrate under continuous exposure to hexenolactone.

The decrease in sensitivity during the course of development was apparent also from the behavior of eggs and embryos exposed to 1.0 mg% hexenolactone. Those exposed at St. 2 or 3 went through the first three cleavages at the normal rate, as mentioned above, but thereafter cell division was strongly inhibited and development stopped altogether after 8 to 12 hours in the hexenolactone. By comparison, embryos placed in the chemical at the beginning of gastrulation continued to develop for 4 or more days though morphogenesis was retarded and maximal development corresponded to an abnormal tail-

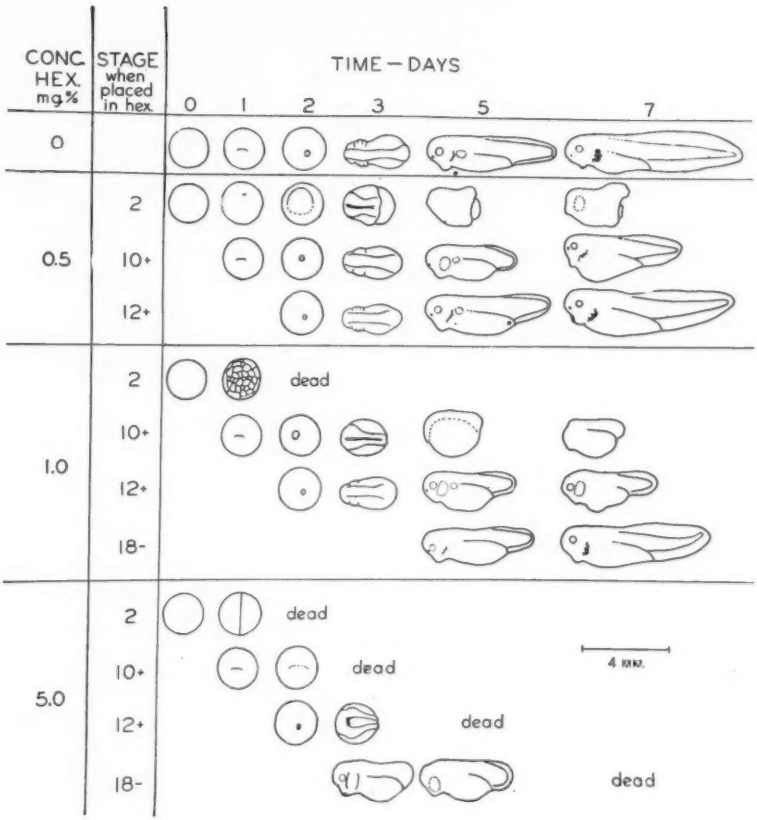

Figure 1

Camera lucida outline drawings of embryos exposed continuously to hexenolactone. Summary of 8 experiments involving a total of 1067 embryos. Embryos exposed to 0.1, 0.05, and 0.01 mg% hexenolactone developed as well as did the controls and are not represented in this figure.

bud stage. Embryos placed in 1.0 mg% hexenolactone at the end of gastrulation or at tailbud stage developed considerably better, though growth and morphogenesis were still definitely retarded, as shown in figure 1.

The effects of 0.5 mg% hexenolactone applied to the eggs at advancing stages of development are also summarized in figure 1. Here too the embryos seem to be much more sensitive in the early stages of development. When exposure was begun shortly after fertilization the embryos were severely retarded during gastrulation and developed into abnormal tail bud stages only. When the embryos were placed in the chemical at the beginning of gastrulation or at later stages development was much more complete.

Hexenolactone in concentrations of 0.1 mg%, 0.05 mg%, and 0.01 mg% were tested for effects on development at all of the stages mentioned above but produced no detectable changes in form or growth.

2. Internal morphology: In view of the fact that hexenolactone is said to be differentially inhibitory for mesenchymal cells of adult animals it was desirable to examine sections of treated embryos for evidence of special effects on the differentiation of mesodermal structures relative to the effects on ectodermal and endodermal ones. Accordingly sections were made of all of the types of specimens shown in figure 1 with the exception of early cleavage stages, which will be dealt with later. The following description is based on an examination of serial sections of 39 embryos.

In blastula and gastrula stages there was clearcut evidence of differential inhibition of cleavage in the vegetal hemisphere. In neurula stages both endoderm and medullary plate showed more disorganization under the influence of hexenolactone than did the remainder of the embryo. Both tissues contained rounded-up cells with altered nuclei, as described below. During post-neurula development there were no striking differential effects but rather a general inhibition of organ development, as illustrated in figure 2. Mesodermal structures such as pronephroi, notochord, myotomes, and mesenchyme were about as well differentiated as ectodermal ones such as brain. eye, nasal organ, and otic capsule. In fact, after a certain degree of differentiation was attained in brain and eye these organs became specially liable to undergo certain cytological alterations (see below).

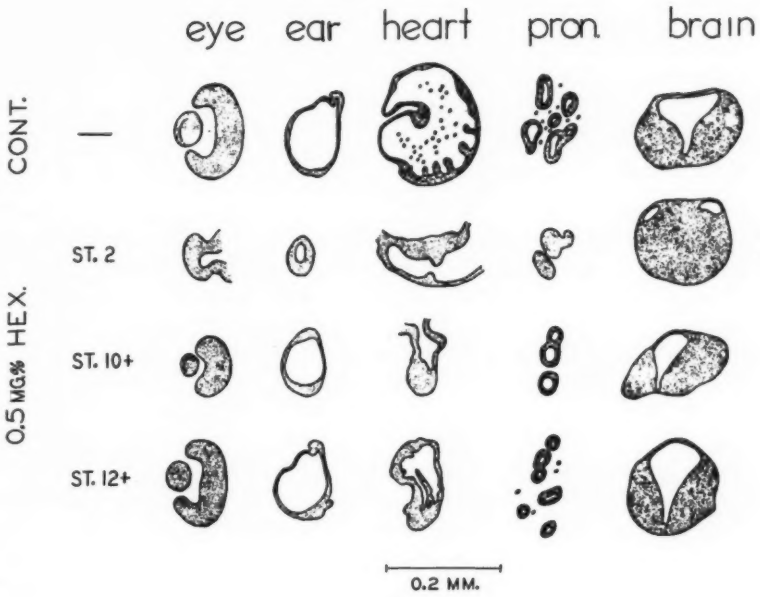

FIGURE 2

Camera lucida drawings of comparable sections through various organs of embryos exposed continuously to 0.5 mg% hexenolactone beginning at St. 2, St. 10+, and St. 12+. The embryos are those illustrated in the upper four drawings in the 7 day column of figure 1. Pron = pronephros; Brain = hindbrain at the level of the pronephros.

However, one set of mesodermal structures, the heart and gills, did appear to be specially inhibited or damaged. The gills, when present, were stubby and in section were seen to have thickened walls, a very small lumen or none at all, and no blood cells. The heart was present as a rudiment or as a tube lacking definite differentiation into the characteristic parts. It also contained no blood cells, presumably because the channels in the gills were not open, for there were numerous blood cells in the blood islands and some of these were seen in the sinus portion of the heart in the less severely inhibited embryos.

It should be noted in passing that there was no sign from these experiments of any special effect of hexenolactone on the neuralizing activity of the chorda-mesoderm. Inhibited embryos that were able to gastrulate, even partially, and to live a few hours thereafter also

succeeded in forming medullary plate and folds. However, the medullary tissue, once it was differentiated, was especially sensitive to hexenolactone—as noted above.

3. Cytology: For the purposes of the present investigation our main interest was in changes occurring in the nucleus. These varied depending upon the stage of development of the embryos when they were exposed to the hexenolactone.

Blastulae: (placed in 1.0 mg% hexenolactone 1 hour after insemination; cleavage stopped within 8 to 12 hours; fixed at 28 hours). In these embryos none of the nuclei were normal. Changes had occurred in some leading to the formation of compact, intensely Feulgen positive bodies. Nuclei in which chromatin condensation of this sort did not occur were for the most part altered in the opposite way with the chromatin either diffuse or in the form of a fine and rather sparse network. These latter nuclei were usually definitely larger than the normal nuclei of the control embryos. The sections of arrested blastulae showed also that there was definitely a differential inhibition of cleavage in the two hemispheres. The animal hemisphere contained 80 to 100 cells per section compared with 4 cells per section for the vegetal hemisphere. In both hemispheres cells frequently contained 2 or more nuclei.

Gastrulae and Neurulae: (placed in 5.0 mg% hexenolactone at St. 10 and St. 12; development stopped in 8 to 12 hours; fixed 24 to 27 hours after beginning of exposure). All of the nuclei in these embryos appeared to be in abnormal interphase or prophase stages. Some of the interphases were enlarged and contained relatively little chromatin. Figure 3 (h, 1) shows two nuclei of this type. In other nuclei, not represented in figure 3, the diminution of Feulgen positive material had proceeded much farther so that only traces of it remained. The prophasic nuclei, on the other hand, were somewhat smaller than normal and contained thickened chromosomes, some of which were distinctly split (fig. 3 e, f, i). However, the nuclear membrane remained intact and there was no sign of spindle formation or of arrangement of chromosomes on a metaphase plate. The thickest chromosomes appeared in other nuclei to be coalescing to form very compact Feulgen positive bodies, some of which still retained traces of the individual chromosomes (fig. 3 j, k). These bodies may

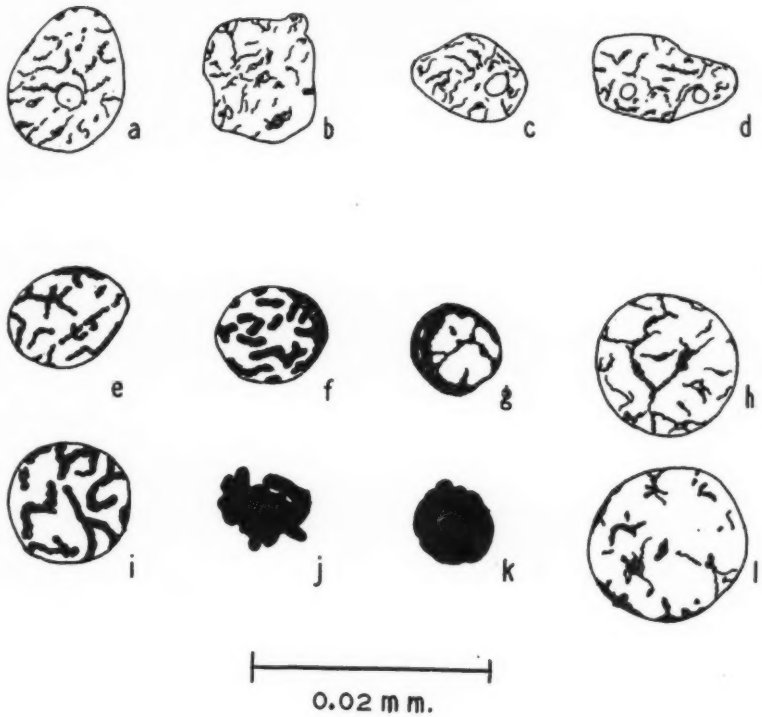

FIGURE 3

a, b, c, and d represent normal nuclei from ectoderm, endoderm, splanchnic mesoderm, and medullary plate of a control embryo at St. 13. The remaining drawings (e through l) illustrate changes in nuclear structure brought about by continuous exposure to 5.0 mg% hexenolactone for 27 hours starting at St. 10. At the time of fixation the embryos were in an inhibited St. 10+. See text for description of nuclei.

also have arisen in a few cases by accumulation of chromatin on the nuclear membrane (fig. 3 g).

Late tail bud—St. 18: (placed in 5.0 mg% hexenolactone at St. 18—, developed for about 16 to 20 hours, fixed at 25 hours). Here the cytological effects were quite different from those described above. The majority of the tissues of the late tail bud embryo displaced neither the enlarged pale nuclei nor the large number of prophase nuclei seen in earlier stages of development. Instead, the majority

of nuclei were in an apparently normal interphasic condition, with an additional few in prophase. The compact Feulgen positive bodies were seen in all tissues but were particularly numerous in brain and eyes. In these organs there were also nuclei with chromatin condensed on the nuclear membrane and it appeared that this form of condensation was the usual mode of origin of the Feulgen positive bodies while origin through coalescence of prophase chromosomes, as described above, occurred relatively infrequently if at all (fig. 4).

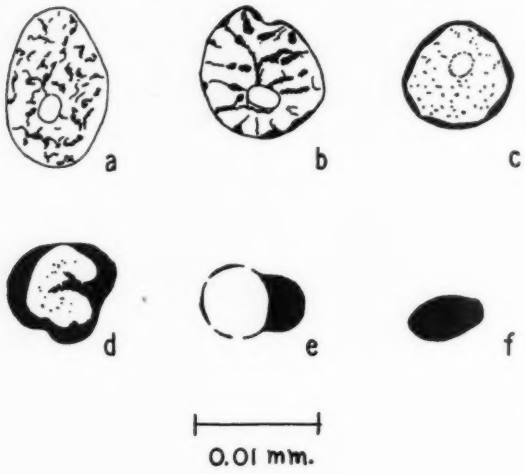

FIGURE 4

Nuclei from the mid-brain of an embryo exposed for 3 days to 1.0 mg% hexenolactone. Exposure begun at St. 12+. At time of fixation the embryos were at St. 18+. Figure a is an approximately normal interphase. Figures b through e show probable successive stages of condensation of chromatin on the nuclear membrane. Figure f shows a small intensely Feulgen positive body of which there were many in brain and eyes.

The endoderm in these animals contained some swollen, pale interphase or early prophase nuclei; it also contained here and there small groups of chromosomes loose in the cytoplasm.

Stages 18 to 22: (embryos placed in 1.0 and 0.5 mg% hexenolactone at stages 2, 10, 12; fixed after developing to St. 18-22). In these embryos the outstanding cytological alterations were in the brain, cord, eye, and endoderm. In the first three organs there appeared, follow-

ing exposure to hexenolactone, large numbers of the compact oval or spherical Feulgen positive bodies. These appeared to arise through condensation of chromatin on the nuclear membrane as already described for the stage 18 embryos exposed to 5.0 mg% hexenolactone (see fig. 4). However, in the present cultures, which were exposed to weaker concentrations of the chemical, the condensed nuclei occurred always in the organs mentioned, and very seldom in other tissues. The other point of interest was that it was apparently necessary for the CNS and eye to differentiate into quite well formed structures before they became especially liable to this nuclear alteration. Thus, animals exposed to 0.5 mg% hexenolactone from St. 2 onward differentiated rudimentary eyes and brain (fig. 2) which contained only a few Feulgen positive bodies, while embryos that were placed in the same concentration of the chemical first at St. 12 differentiated eyes and brain were almost normal in gross appearance but which contained large numbers of the condensed nuclei.

Nuclei in other organs, with the exception of endoderm, appeared to be normal. The endoderm, especially the posterior part of it, contained a variable number of enlarged nuclei that appeared to develop from the interphase.

4. Attempted reversal of cleavage inhibition: One of the most striking cytological features described above was the occurrence of considerable numbers of prophasic nuclei in gastrulae and neurulae exposed continuously to 5.0 mg% hexenolactone for approximately 24 hours. In some of these nuclei the chromosomes were definitely split, but they never aligned themselves on a metaphase plate and never showed signs of anaphase movement. It was thought possible that if the inhibition could be reversed there might ensue changes in chromosome number, and for the purpose of changing the ploidy of the whole embryo it was decided to attempt to bring about the reversal in early cleavage stages.

Eggs (diploid and haploid) were placed in 5.6 mg% hexenolactone[2] at the beginning of first cleavage. At 20 minute intervals thereafter some of the eggs were removed from the chemical, washed thrice, and then were observed as they developed in 10% Ringer's

[2]This concentration of hexenolactone (= 5.0 X 10^{-4} molar) has very nearly the same biological effects as the 5.0 mg% solution and was used to make comparisons with work on other inhibitors easier.

solution. Some of these were fixed about 24 hours later. Sixteen embryos were sectioned serially and studied to determine the condition of the nuclei.

The results are summarized in table 2. Exposures of 20 minutes

TABLE 2

EFFECTS OF LIMITED EXPOSURES OF EGGS TO HEXENOLACTONE ON THEIR SUBSEQUENT DEVELOPMENT

Concentration of test solution 5 X 10^{-4} molar (5.6 mg%). Exposure begun at time of appearance of first cleavage furrow, 2½ hours following insemination. Samples removed thereafter at the time intervals listed. Normal development as mentioned in the table means development to stage 25 for diploids and to the corresponding stage for haploids.

Time-minutes. Eggs in 5 X 10^{-4} Molar hexenolactone	Number of eggs		Development (diploids and haploids)
	Diploid	Haploid	
0 (control)	63	16	Normal
20	14	7	Slightly retarded. Otherwise normal.
40	15	7	Retarded. Otherwise normal.
60	15	7	Animal half of egg divided into small cells. Vegetal half divided into 4 large cells only.
80	15	7	Small disc of cells at animal pole. Main part of egg not cleaved beyond 4 cell stage.
100	16	7	A few abortive cleavages at animal pole. Main part of egg divided into 4 cells only.
120	16	5	Not cleaved beyond 4 cell stage.
180	33	2	Not cleaved beyond 4 cell stage.

or 40 minutes had no effect on subsequent development other than a slight and temporary retardation. Sixty minutes exposure suppressed cleavage of the vegetal hemisphere almost completely while allowing the animal hemisphere to cleave normally. These eggs later failed to gastrulate and formed only a crinkled cap of ectoderm sitting on a mass of uncleaved yolk. In eggs exposed for 60 minutes and fixed 27 hours after removal from hexenolactone, the nuclei of the animal hemisphere had the normal appearance (fig. 6 e, f). In the vegetal

hemisphere there were numerous nuclei even though cytoplasmic cleavage had not gone beyond the 4 cell condition. However, the majority of these nuclei were enlarged and abnormal. Some of them consisted of a fine Feulgen positive threadwork in a relatively large amount of Feulgen negative "nucleoplasm" (fig. 6 i). Others, also enlarged, consisted of Feulgen positive material dispersed more uniformly through the body of the nucleus (fig. 6 g, h). Finally, there were some tremendously enlarged nuclei containing varying amounts of Feulgen positive material in the form, usually, of a fine threadwork (fig. 6 j). Nuclear changes similar to these apparently occur also in the uncleaved yolk of centrifuged (O. Hertwig, 1897) or colchicine treated (Keppel & Dawson, '39) frog embryos.

In eggs exposed for 80 minutes to the hexenolactone cleavage was restricted to a small disc of cells at the animal pole. These contained

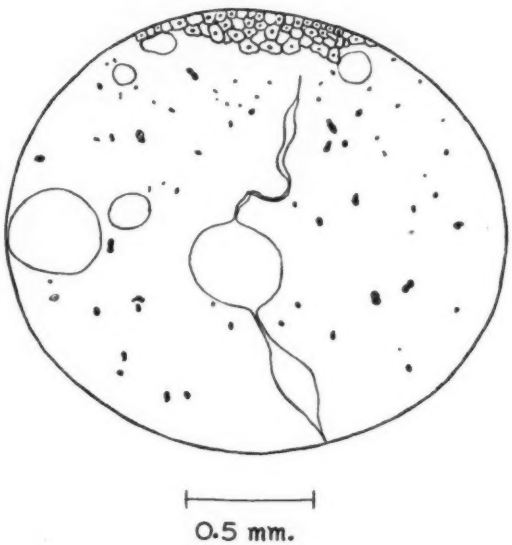

FIGURE 5

Section running through the animal-vegetal axis of an egg that had been given an 80 minute exposure to 5.6 mg% hexenolactone at St. 3. After removal from the hexenolactone the egg developed for 25 hours in 10% Ringer's solution before it was fixed. For description see text.

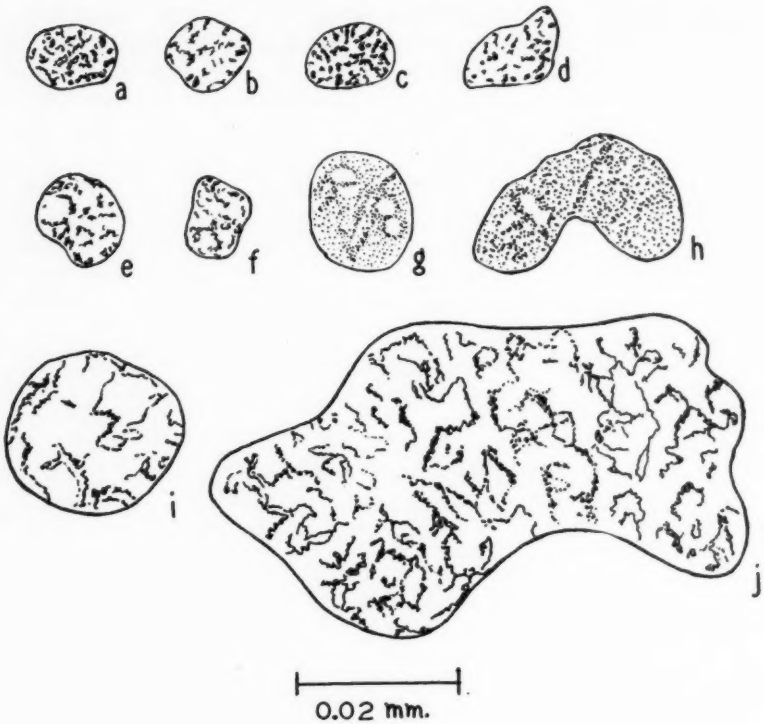

FIGURE 6

Drawings of nuclei from the egg shown in figure 5. Figures a through d represent nuclei from a control embryo (early gastrula). Figures e and f are apparently normal nuclei from the small animal pole cells of the treated egg. Figures g through j show abnormal nuclei from the uncleaved part of the same egg.

apparently normal nuclei. The remainder of the egg was uncleaved beyond the 4 cell stage and contained numerous abnormal nuclei as described above. It also contained a few small or medium sized vesicles (fig. 5).

Eggs exposed 100 minutes showed externally only 2 cleavage furrows. Sections revealed in addition a group of a few small cells located on the inner surface of a blastomere and projecting into the enlarged space between blastomeres. These cells contained nuclei

of approximately normal size and appearance. The main part of the egg was divided into 4 cells only and contained many of the bizarre nuclei already described.

After 120 minutes exposure there was no cleavage beyond the 4 cell stage. Sections of the eggs fixed 24 hours after removal from the hexenolactone revealed a large vesicle in the animal hemisphere. In the fixed specimens only two cleavage furrows could be made out, but there were many of the abnormal nuclei. Most of these were located in an area just above the equator of the egg.

Eggs exposed 140 minutes divided into 4 cells only, formed no vesicles, and contained only a few small nuclei near the animal pole. These were surrounded by so much melanin that it was difficult to make out their structure.

Finally, eggs exposed for 180 minutes consisted of 4 cells as above, but contained very few nuclei—about 6 per egg. These were small, compact structures staining intensely with the Feulgen reagent.

From these experiments it appears that any exposure to hexenolactone that was adequate to stop cleavage was irreversible. Such irreversibly inhibited eggs contained altered nuclei, as described above, while eggs given lighter exposures, insufficient to stop development, contained normal nuclei. The altered nuclei were frequently enlarged and many of them contained more than the normal amount of Feulgen positive material; but none showed any signs of being able to undergo mitosis. Considering these facts it seemed unlikely that increase in ploidy could be produced in viable eggs by hexenolactone. However, the action of the chemical was interesting from other points of view. These experiments showed definitely that the vegetal hemisphere was more sensitive than the animal hemisphere, and demonstrated that nuclei could divide in cytoplasm that was completely inhibited from cleaving.

II Differential Effects of Hexenolactone on Haploids and Diploids

The experiments described in the preceding section of this report have established the following facts about the effects of hexenolactone on diploid embryos:

1) The embryos are highly sensitive to the chemical during early stages of development and become less sensitive in later stages.

2) The vegetal hemisphere of the egg is more sensitive than the animal hemisphere.
3) Nuclear division in cleavage and blastula stages of development can take place without cytoplasmic division.

These facts can be fitted together if it is assumed that the cytoplasm is much more sensitive to hexenolactone than is the nucleus. Then as development proceeds and the amount of nuclear material increases, the sensitivity of the embryos should decrease—as we find that it does. Also, on this assumption the vegetal hemisphere, containing as it does large cells with nuclei of about the same size as the animal cell nuclei, should be more sensitive than the animal hemisphere—which again is what we find. Furthermore, if this interpretation regarding the relative sensitivity of cytoplasm and nucleus is correct we should find differences in sensitivity between haploids and diploids as soon as there is any difference between the two in total amount of nuclear material per egg.[2a] Such a difference would presumably become manifest sometime during gastrulation so far as we can predict from the quite meagre existing information on cell number of diploids and haploids (G. Hertwig, '13). This information suggests that haploid early gastrulae contain about twice as many cells as diploids at the same stage, while in post-gastrula stages the haploid/diploid cell number ratio is reduced.

In order to test the relative sensitivity of haploids and diploids to hexenolactone we had to have fairly large numbers of haploids. These were made according to Porter's ('39) procedure. The dependability of this technique can be judged by reference to table 3, which shows that 94% of 911 eggs operated on for removal of the second polar body spindle actually turned out to be haploids. Parenthetically it should be remarked that the operations were practically always done quickly so as to provide, for any given experiment, ade-

TABLE 3

SUMMARY OF DEVELOPMENT OF EGGS OPERATED ON FOR REMOVAL OF SECOND POLAR BODY SPINDLE

Total number of eggs operated on	Number developing as typical haploids	Number developing as typical diploids	Questionable	% developing as haploids
911	854	54	3	94%

[2a] In this argument diploids and haploids are regarded as having the same total amount of cytoplasm per egg. This is approximately correct during early development, at least.

quate numbers of haploids all inseminated at approximately the same time.

The criteria for haploidy were as follows: 1) Small size of cells as seen in the living embryos. 2) Regularly delayed development from the beginning of gastrulation onward. 3) Development of the characteristic syndrome of morphological abnormalities as described by Porter and others. 4) Sample chromosome counts.

For the cytological studies I am indebted to Dr. Jack Schultz who prepared and examined the material upon which the data given in table 4 are based. Parts of embryos, as listed in the legend to

TABLE 4

CYTOLOGICAL ANALYSIS OF HAPLOIDS AND DIPLOIDS

Material for the smears was taken from embryos ranging in stage from early gastrula (St. 10) to St. 24 for diploids and from the corresponding stages for haploids. Smears were made of dorsal and ventral ectoderm of gastrulae, and from eye, brain, pronephros, tail, and liver of post-neurula embryos. In each preparation chromosomes were counted in at least 2 or 3 metaphase figures. Observations were made also on the size of resting nuclei and the number of nucleoli. In the sectioned material the observations were limited to measurements of nuclear size and determination of nucleolar number.

Category according to external appearance	Number of embryos	Type of preparation	Number of preparations	Ploidy found
Haploid	14	Sections	14	N
Haploid	25	Smears	64	N
Diploid	15	Smears	46	2N
Questionable, probably diploid	1	Smears	3	2N

table 4, were squashed in acetic-orcein and mounted permanently according to the method of LaCour ('41). In these preparations the chromosomes were stained red or pink while the yolk was left colorless, or stained green when fast green counterstain was used.

The haploid number of chromosomes was determined to be 13, in keeping with other counts recorded in the literature. The chromosome counts showed that all embryos unqualifiedly classified as haploid on the basis of external appearance turned out actually to have a single set of chromosomes. There was some regulation to diploidy in some of the older embryos, but this occurred in only a small proportion of cells.

1. Experiments on eggs and sperm: (Separate exposure of nu-

cleus and cytoplasm to hexenolactone). In section I of this report it was shown that nuclear division had apparently taken place in cytoplasm that was completely inhibited from cleaving. This indicated that the cytoplasm was differentially susceptible to hexenolactone, as noted above. To test this interpretation nucleus and cytoplasm were exposed separately to the chemical in the following ways (see table 5).

TABLE 5

ANALYSIS OF THE EFFECTS OF HEXENOLACTONE ON CYTOPLASM AND NUCLEUS OF R. PIPIENS EGG

Eggs after fertilization were exposed to 5.6 mg% (= 5 X 10^{-4} M.) hexenolactone for 2 hours. Eggs before fertilization were exposed for 1 hour to the same concentration. Sperm were exposed to 5.6 mg% or to 16.8 mg% hexenolactone for 2 hours. See text for additional details concerning development of eggs.

Treatment of	Components exposed to hexenolactone			Number of eggs	Result
	Egg cyt.	♀ nucleus	♂ nucleus		
Fertilized diploid	+	+	+	288	Development stopped
Fertilized egg—egg nucleus removed	+		+	113	Development stopped
Unfertilized egg; fertilized with normal sperm	+	+	—	174	Development stopped
Unfertilized egg; fertilized with normal sperm. Egg nucleus removed	+		—	39	Development stopped
Sperm only	—	—	+	360	Normal diploid development
Sperm only; egg nucleus removed	—		+	51	Normal haploid development

a) Concentrated sperm suspensions were made up by macerating single testes in 1.5 ml of 5.6 or 16.8 mg% hexenolactone. The volume of the individual testis was less than 0.1 ml so that even if it were all water the minimum hexenolactone concentration would still be greater than that known to inhibit completely the cleavage of fertilized eggs within 2 hours (see table 1). The sperm were left in the strong hexenolactone for 2 hours. The suspensions were then diluted 25 fold giving a maximum concentration of 0.67 mg% hexenolactone in the inseminating suspensions. Fifteen minutes after insemination the eggs were washed with 10% Ringer's solution. The subsequent development of these aggs was perfectly normal.

b) After insemination of normal eggs with treated sperm as described under a) the second polar body spindle was removed leaving in the egg the treated male nucleus only. These eggs developed as typical haploids proving that the sperm nucleus was entirely unaffected by the treatment with hexenolactone.

c) Eggs were exposed to 5.6 mg% hexenolactone in Ringer's solution for 1 hour before insemination. They were then inseminated in the usual way with normal sperm in 10% Ringer's solution. The majority of these eggs rotated and later formed an abortive first cleavage furrow, but did not develop further. Control eggs exposed to Ringer's solution for 1 hour before insemination developed normally.

d) Eggs exposed to hexenolactone as described above were then, after 1 hour, removed to 10% Ringer's solution and inseminated with normal sperm. About 30 minutes after insemination the eggs began to rotate and the position of the 2nd polar body spindle was clearly indicated by the withdrawal of pigment from the egg surface just above it. Polar body spindles were removed from a number of eggs. These were then made up of treated cytoplasm containing only untreated male nucleus. Again the development proceeded only to an abortive first cleavage just as in c) above. In other words, when the cytoplasm alone was treated with hexenolactone the inhibition was just as severe as it was when the treatment was given to cytoplasm plus female nucleus, or cytoplasm plus female plus male nuclei. Control eggs that were exposed to Ringer's solution for 1 hour, then inseminated and operated on for removal of the second polar body spindle, developed as typical haploids.

These experiments show quite conclusively that hexenolactone inhibition of cleavage is due primarily to effects on the cytoplasm.

2. Continuous exposure of haploids and diploids to hexenolactone: In this set of experiments the haploids and diploids were inseminated and prepared in the usual way and then placed in the hexenolactone solutions at the time of first cleavage, or at the beginning of gastrulation. The development of these embryos is summarized in figure 7 and table 6.

Exposure begun at stage 3 (2 cells); It will be seen from table 6 that concentrations of 5.0 and 1.0 mg%, which stopped the development of diploids in pre-gastrula stages, had the identical effect on

TABLE 6

SUMMARY OF EXPERIMENTS ON EFFECTS OF CONTINUOUS EXPOSURE TO HEXENOLACTONE ON DIPLOID AND HAPLOID DEVELOPMENT

Conc. hex. mg/100 ml	Stage when eggs placed in hex.	Number of eggs		Development to		Exposure time required to stop development	
		Diploid	Haploid	Diploid	Haploid	Diploid	Haploid
5.0	St. 3	100	68	St. 4 or 5	St. 4 or 5	2 hrs.	2 hrs.
1.0	St. 3	32	32	St. 7.5 or 8	St. 7.5 or 8	8 to 12 hrs.	8 to 12 hrs.
0.75	St. 3	7	7	St. 8.5	St. 8.5	——	——
0.5	St. 3	39	39	St. 18 abn.	St. 14 abn.	6 to 7 days	3 days
0.1	St. 3	20	20	No effect	No effect	——	——
Controls		74	64	Diploid normal dev.	Normal haploid dev.		
5.0	St. 10	45	26	St. 10+	St. 10+	8 hrs.	6 hrs.
1.0	St. 10	21	21	St. 17.5 abn.	St. 14.5 abn.	4 to 6 days	2 days
0.5	St. 10	9	9	Ca. St. 20 abn.	Ca. St. 18 abn.	——	——
0.2	St. 10	9	9	Only sl. retard	Only sl. retard	——	——
Controls		28	28	Normal dev.	Normal dev.		

haploids. However, 0.5 mg%, which allowed development to go on to post-gastrula stages, had a definitely differential effect. In this solution the haploids failed to complete gastrulation and formed no neural structures or only abortive ones. Development was stopped in three days and the embryos began to disintegrate on the fourth day. On the other hand, the diploids developed to an abnormal stage 18 and were still intact at the end of the experiment after 6 days of exposure.

Exposure started at the beginning of gastrulation (St. 10): In these experiments all concentrations of 0.5 mg% or above had differential effects on haploids and diploids. In diploids exposed to 5.0 mg% hexenolactone the formation of the lateral lips of the blastopore continued slowly for about 8 hours and then stopped. In the same concentration the haploids formed the lateral lips even more slowly and stopped developing within about 6 hours.

In 1.0 mg% hexenolactone the diploids completed gastrulation and formed an abnormal late tail bud stage before development stopped

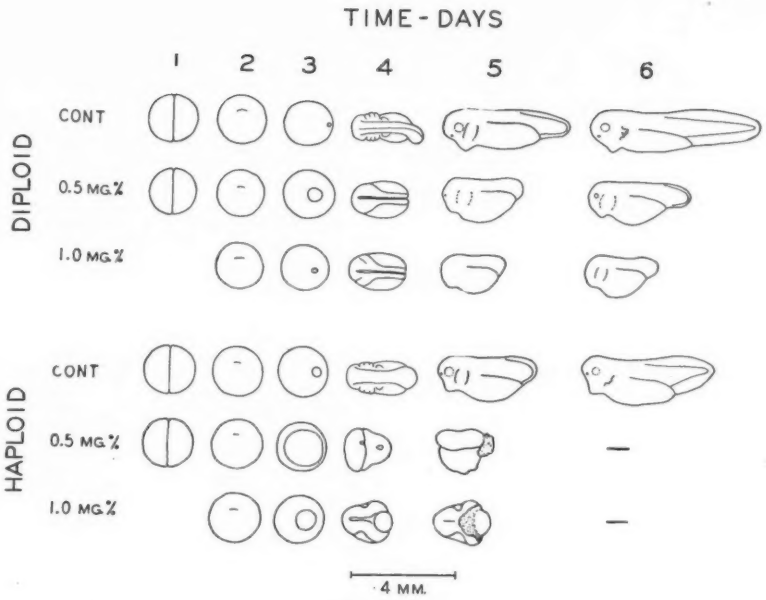

FIGURE 7

Illustrating the relative effects of hexenolactone on diploid and haploid embryos. Eggs were placed in the 0.5 mg% solution at the two cell stage, and into the 1.0 mg% solution at the beginning of gastrulation. Stippled areas on the haploid embryos represent disintegrating tissue.

after 4 to 6 days in the chemical. By comparison the haploids failed to complete gastrulation and formed only an abortive neurula with large persistent yolk plug. Development was stopped in 2 days and disintegration of the neural tissues began on the third day.

A concentration of 0.5 mg% hexenolactone also had a differential effect on the development of haploids and diploids but the exact limits of development were not worked out. A concentration of 0.2 mg% had only a slightly retardative effect which was not clearly different for haploids and diploids.

These experiments indicate that before gastrulation haploids and diploids are equally affected by the hexenolactone while after the beginning of gastrulation the haploids become the more sensitive.

3. Reversal of inhibition of haploids and diploids: In order to obtain more accurate information on the relative sensitivity of haploids and diploids we did a number of reversal experiments. In these we determined the exposure time (to 5.6 mg% hexenolactone) necessary for complete and irreversible inhibition at different stages of development. The results (table 7) confirm the findings from the

TABLE 7

SENSITIVITY OF DIPLOIDS AND HAPLOIDS TO HEXENOLACTONE AT DIFFERENT STAGES OF DEVELOPMENT

Two or three experiments were done at each stage of development listed, except for stages 7.5 and 8 at which single experiments were done, and stages 9 and 20 at which two experiments were done with diploids and one with haploids. At the beginning of each experiment the diploid and haploid embryos (30 to 60 each) were placed in the hexenolactone. Thereafter samples of 7 to 15 embryos from each culture were removed from the chemical at regular intervals. (20 minutes for cleavage stages, and 1 hour for all later stages).

Stage	Number of eggs		Hours exposure to 5×10^{-4} M hexenolactone required to stop development	
	Diploid	Haploid	Diploid	Haploid
Unfertilized egg	174	39	1	1
St. 3 (one to two cells)	198	93	2	2
St. 7.5 (young blastula)	95	63	3	3
St. 8— (Mid. blast)	33		4-5	
St. 9 (late blast)	160	70	5	5
St. 10 (dorsal lip)	199	94	8	6
St. 12 (yolk plug)	169	94	13	10
St. 16 (neurula)	118	102	22+	18
St. 20 (ext. gills)	133	44	18	14 (or less)
St. 24 (tad)	110	—	7	—

continuous exposure experiments. In pre-gastrula stages the exposure time for complete inhibition was the same for haploids and diploids; after the beginning of gastrulation the haploids became the more sensitive, i.e. were completely inhibited by shorter exposures. In addition the figures in table 7 show for both haploids and diploids a steadily decreasing sensitivity reaching a minimum at neurula stage. Thereafter the sensitivity to hexenolactone increased again, this increase being correlated with the onset of damage to the developing gills, heart, and CNS, as described in Part I of this report.

In connection with the reversal experiments, sections were made of 14 partially inhibited embryos. In most cases the embryos (haploids and diploids) were exposed to 5.6 mg% hexenolactone for roughly one-half the time necessary to produce complete stoppage of devel-

opment, as follows: St. 10, 4 hours; St. 12, 9 hours; St. 16.5, 10 hours; St. 19—, 9 hours; St. 20, 10 hours. After being exposed to the hexenolactone the embryos were placed in 10% Ringer's solution for 15 hours and then were fixed. The sections showed that the vast majority of cells in all experimental embryos were apparently in normal interphasic condition. In gastrulae and neurulae, both haploid and diploid, there appeared to be more than the normal number of metaphase nuclei, and in later stages there were a few condensed nuclei in CNS and eyes. Otherwise there were no cytological effects even though the development of the embryos had been severely retarded. This suggests that the cytological alterations observed in completely inhibited embryos are largely sequelae of inhibition, the primary effects having already occurred in cells that still appear under the microscope to be normal.

4. Relative effects of hexenolactone on the growth in length of diploid and haploid embryos: In three of the reversal experiments described above the total length of the embryos was measured after two to four days had elapsed following the exposure to 5.6 mg% hexenolactone. In two additional experiments haploid and diploid embryos were placed in individual dishes each containing about 5 ml

TABLE 8

SUMMARY OF OBSERVATIONS ON GROWTH (INCREASE IN LENGTH=ΔL) OF EMBRYOS EXPOSED TO HEXENOLACTONE BEGINNING AT ST. 16 TO ST. 18

The percentage figures given in the right hand column show that the growth of haploids was consistently more inhibited than was that of the diploids. In two experiments (No. 26 and No. 29) statistical analysis showed that this difference between $\Delta L_{hex}/\Delta L_{cont}$ for haploids and diploids was significant, d/σ_d being approximately 3.

Exp.		No. embryos		$\Delta L_{hex}/\Delta L_{cont}$ X 100	
No.	Treatment	Dip.	Hap.	Dip.	Hap.
11	8 hrs. in 5.6 mg% hex. Measured 2 days after exposure	42	41	50%	28%
24	8 hrs. in 5.6 mg% hex. Measured 2 days after exposure	59	29	65%	58%
26a	12 hrs. in 5.6 mg% hex. Measured 2 days after exposure	41	24	71%	41%
29	Continuous exposure to 1.12 mg% hex. for 2 days	15	15	64%	56%
29	Continuous exposure to 2.24 mg% hex. for 1 day	15	15	81%	61%
26	Continuous exposure to 0.56 mg% hex. for 2 days	12	12	87%	81%
26	Continuous exposure to 1.12 mg% hex. for 2 days	12	12	70%	59%

of solution. The experimental embryos were exposed continuously to 0.56, 1.12, or 2.24 mg% hexenolactone over a period of 2 to 3 days during which time daily measurements of total length were made. The results are summarized in table 8, from which it can be seen that the growth of the experimental haploids relative to the haploid controls was consistently smaller than was the growth of the experimental diploids relative to their controls. Another way of expressing the results is to calculate the ratio, haploid growth/diploid growth, for control and experimental cultures. Daily length increments of haploids and diploids were treated in this way and plotted against time with the result shown in figure 8, from which it is again apparent that the haploids were more inhibited than the diploids.

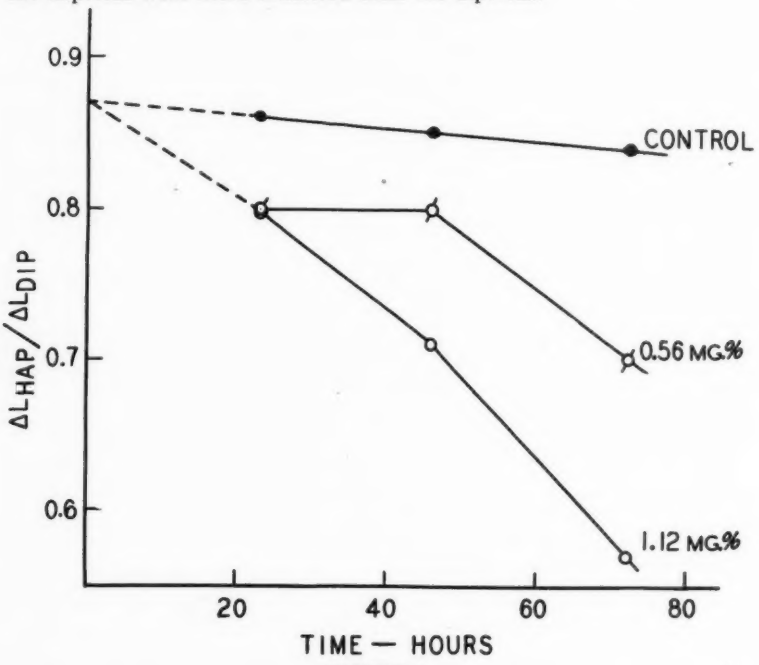

FIGURE 8

Illustrating the differential effect of hexenolactone on the growth rate (ΔL=daily length increment) of haploids and diploids. Hexenolactone is seen to reduce the proportional growth of the haploids with respect to that of the diploids. Summary of experiment 26 in which 24 haploid and 24 diploid tailbud embryos were exposed continuously to hexenolactone for 3 days.

Discussion

It has been known for some time that certain extracts of malted grains, Sorbus berries, and other natural products act as reversible growth inhibitors with the property of being more effective in suppressing the growth of mesenchymal cells than that of epithelial ones (Heaton, '26, '29; Medawar, '37). From more recent studies it appears that the active substance in these extracts is hexenolactone, and it is stated that this chemical likewise acts as a differential inhibitor of mesenchymal cell growth (Medawar et al, '43; Kuhn et al, '43; but not confirmed by Royle, '45). Whether it would also suppress specifically the differentiation of mesenchyme and other mesodermal structures in embryos was one of the questions we sought to answer in the present investigation. Our results do not reveal any such action of hexenolactone. In the early stages of development the vegetal (future endodermal) part of the embryo is especially sensitive to the chemical. In gastrulae and neurulae the endoderm and medullary plate are more disorganized than other tissues. During post-neurula development the heart and gills are particularly sensitive, but other mesodermal tissues, including mesenchyme, are not. The central nervous system and eyes also give evidence of being severely damaged by hexenolactone, while ectodermal organs otherwise are very little affected. Thus the experiments demonstrate that there is no striking differential effect on any one class of structures, but rather that certain derivatives of all germ layers are damaged or inhibited.

The cytological changes produced in the structures mentioned above are frequently quite striking, but they occur mainly in irreversibly inhibited embryos and presumably represent only end products of inhibition.

The cytological study of eggs inhibited in early cleavage stages is none the less valuable in demonstrating that the nucleus can divide in a completely inhibited cytoplasm. This result, together with the results of experiments in which cytoplasm and nucleus were treated separately and put together in various combinations, allows us to conclude that the cytoplasm is much more sensitive than the nucleus to hexenolactone inhibition during the earliest stages of development. It is at this time that the sensitivity of the egg is greatest. As the

embryo develops and the number of nuclei increases the sensitivity to hexenolactone decreases.[3] In pre-gastrula stages this decrease occurs to the same degree in haploids and diploids. After the beginning of gastrulation the decrease continues for both, but at different rates such that the haploids become more sensitive than the diploids. In view of the studies on unfertilized eggs and early cleavage stages it seems reasonable to attribute the general decrease in sensitivity to an increase in some nuclear component or derivative which gradually comes to assume a more important role in development and which is considerably less sensitive to hexenolactone than is the cytoplasmic counterpart. This implies that the nuclear component involved is present in the same form and amount in pre-gastrula haploids and diploids, and that it becomes restricted in amount or changed in form in haploid gastrulae and neurulae as compared with diploids at the same stages.

What this component might be is quite conjectural, and it is natural, therefore, to speculate on the possibility that it might represent one or several enzymes. With regard to effects on frog embryos hexenolactone is about as active as many enzyme inhibitors, which retard or suppress development in concentrations of 10^{-3} to 10^{-5} molar. As was noted above, hexenolactone is most effective in inhibiting cleavage and is much less inhibitory for later stages. Thus its activity places it in the category of dehydrogenase inhibitors like phenyl urethane (Brachet, '34) and chloretone (Moog, '44) which have the same general type of effect on frog development. Leastwise hexenolactone cannot produce its effect on early development through inhibition of oxidases requiring molecular oxygen for we know that cleavage continues in a completely anaërobic atmosphere and in the presence of cyanide (Brachet, '34; Spiegelman and Moog, '45).

There is another reason for thinking that hexenolactone may affect primarily the dehydrogenase component of enzyme systems. From the report of Hauschka et al ('45) we know that the lactone combines with —SH amino acids. It is possible therefore that it may combine also with protein —SH groups. If so it would be, as already suggested by Hauschka et al, a possible inhibitor of a large number

[3]The increase in sensitivity to hexenolactone during later development (St. 20 onward) is not considered in this discussion. It is probably the result to damage to one or more vital organs such as heart, gills, or central nervous system.

of enzymes that have been shown by Barron and Singer ('45) and Singer and Barron ('45) to require —SH in the activating protein (dehydrogenase) part of the enzyme complex.

In this connection it should be noted that —SH in amphibian embryos increases during development and is probably involved in morphogenesis (Brachet, '38; Brachet and Rapkine, '39)[4]; and that —SH is certainly important in controlling cell division (Hammett, '29, '42; Voegtlin and Chalkley, '30; and others). Strong concentrations of Na monoiodoacetate (M/30, M/60), which block —SH groups, allow frog eggs to cleave but suppress gastrulation (Brachet, '38). This is taken to indicate that —SH proteins begin to play an important role during gastrulation. If this is correct, i.e. if iodoacetate inhibits by specifically binding essential protein —SH groups[5], it becomes difficult to interpret the action of hexenolactone on the same basis. This mode of action is not precluded inasmuch as hexenolactone is considerably more effective in stopping gastrulation than is iodoacetate. But the still greater effect of the lactone on pre-gastrula embryos would have to be due to combination either with —SH groups not affected by iodoacetate, or with some other system entirely—for example the phosphoprotein phosphatase of Harris ('44). This enzyme is very active in unfertilized frog egg brei and probably is not an —SH enzyme since it is a part of, or associated with, the yolk platelets which give no evidence of containing fixed —SH (Brachet, '38).

Summary

Hexenolactone is a rather powerful inhibitor of frog development, producing definite retardation of growth and differentiation in concentrations as low as 5×10^{-5} molar. The sensitivity of the embryos (Rana pipiens) to the chemical is greatest at the very beginning of development and decreases gradually during blastula and gastrula stages. In post-neurula embryos sensitivity increases again, probably as a result of damage to vital organs like the heart and central nervous system upon which the embryo comes to depend for its survival.

[4]The work of Brachet and Rapkine ('39), according to which oxidizing solutions (alloxan, oxidized glutathione) inhibit neuralization while reducing ones (cysteine, reduced glutathione) stimulate it, has not been confirmed by Holtfreter ('45).

[5]According to Barron and Singer ('45) iodoacetate may inhibit enzyme activity by a mechanism other than combination with —SH groups; possibly by combination with $-NH_2$ groups.

A study of sectioned material shows that cleavage of the vegetal hemisphere during early development is more inhibited by hexenolactone than is that of the animal hemisphere, and that nuclear division can take place without cytoplasmic cleavage in eggs given appropriate exposures to the chemical. In post-gastrula stages certain derivatives of all three germ layers are inhibited or damaged differentially by hexenolactone.

During development immediately following fertilization hexenolactone appears to inhibit mainly through its effects on the egg cytoplasm. This conclusion is based on the cytological studies mentioned above and on experiments showing: a) that spermatozoa are not affected by hexenolactone, and b) that eggs consisting of hexenolactone treated cytoplasm plus normal nucleus are just as severely inhibited as are eggs in which cytoplasm and nucleus both are treated.

A comparison of androgenetic haploids with normal diploids shows that the sensitivity to hexenolactone decreases at the same rate for both during pre-gastrula development. After the beginning of gastrulation the decrease continues, but at different rates such that the haploids become definitely more sensitive to the chemical. This differential sensitivity applies to both differentiation and growth (increase in length). The implications of these findings are presented in the text.

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A COMPARISON OF REGENERATION RATES AND METAMORPHOSIS IN TRITURUS AND AMBLYSTOMA[1]

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It is well known that metamorphosis has a profound effect on regeneration. In frogs the regenerative power is lost during metamorphosis (Barfurth 1899, Byrnes 1904). In salamanders the ability to regenerate is not lost, but there is a decrease in rate; for instance, it is common knowledge that an Axylotl will regenerate more rapidly than the adult Amblystoma tigrinum.

In many cases of Amphibia, metamorphosis prepares the animal for terrestrial life. Is there something inherent in land animals which retards or inhibits regeneration? The salamander Triturus viridescens is interesting in this respect. In the region of Northampton its early larval life is spent in the water; then for several years it lives on land as a red eft; the adult lives in the water as the common newt. In the vicinity of Wood's Hole, however, the red eft stage is omitted; the adults have an incomplete metamorphosis, do not leave the water, and some retain gill stumps.

It was hoped that by a comparison of Triturus and Amblystona during metamorphosis some light would be thrown on the relationship of metamorphosis to the rate of regeneration and structural changes. The normal and regenerating limbs were studied histologically as well as grossly.

A COMPARISON OF THE RATES OF REGENERATION OF RED EFTS AND ADULT NEWTS

A comparison of the rates of regeneration of red efts and adult newts necessarily involved two variables; age and natural habitat; therefore the animals were arranged in the following three groups.

[1]Contributions from the Department of Zoology, Smith College, no. 210.

Group I 12 red efts and 12 adult newts confined to water
Group II 12 red efts and 12 adult newts kept moist on damp paper
Group III 12 red efts and 12 adult newts placed in an aquarium where they could get out of the water

The animals in each group were paired as to size, similar shades of green and had the same slightly rough skin. They were accustomed to their new habitat before the experiment was begun and all were anesthetised in MS222 when their right hands were amputated at the wrist. The temperature during the experiment averaged about 27°C so that regeneration was quite rapid. The first fingerbuds appeared three weeks after amputation and the average number of days for their appearance is given in Table I. Since a number of the animals living in water were killed by a fungal disease, the number of animals in each group which lived and were used in the analysis appears in parenthesis.

TABLE I
AVERAGE DATES FOR THE FIRST APPEARANCE OF FINGERBUDS

	Group I (water)	Group II (land)	Group III (both)
Greens:	25.7 days (8)	27.8 days (12)	24 days (11)
Reds:	25.3 days (6)	24.2 days (12)	23 days (11)

From the above table there appears to be a slight difference in the rates of regeneration, but the standard deviations for each of the groups are so large that the differences cannot be considered significant.

After three months the amount each limb had regenerated was measured; only groups II and III survived that period. The green salamanders regenerated more in millimeters than the reds of the same size, but the greens had larger left (control) hands in proportion to their bodies, so that at the end of three months each animal had regenerated approximately one half of its hand. There was a .75 correlation between the size of the normal left hand and the amount the right hand had regenerated; there were higher correlations in the individual groups of reds and greens. Only animals with normal regeneration are included in the figures which are given in Table II.

From Table II the writer concludes that the difference in the

TABLE II
AVERAGE AMOUNT REGENERATED AFTER THREE MONTHS

	Group II (land)	Group III (land and water)
Greens:	49%	54%
Reds:	53%	48%

amounts regenerated were not significant, and that there was no difference in the rate of regeneration due to the age of these Triturus as indicated by their color or due to adaptations to habitat. Since the green adult salamanders are several years older than the red efts and yet regenerate as quickly, this experiment indicates that within this range, age does not affect the rate of regeneration.

A COMPARISON OF THE RATES OF REGENERATION OF NEOTENOUS NEWTS AND ADULT NEWTS

Although the previous experiment indicated that the rate of regeneration was not affected by the age of adult Triturus it was thought that immature newts would regenerate more rapidly than adults, therefore the Triturus in this experiment were arranged in the following groups.

Group A from Wood's Hole: 24 adult newts with gills, total length about 80 mm.

Group B from Wood's Hole: 22 small newts with gills, total length about 47 mm.

Group C from the vicinity of Northampton: 13 adult newts without gills, total length about 80 mm.

Contrary to what was expected, all three groups of Triturus got fingerbuds at the same time. There were differences in the individual groups but no one group was ahead of the others. From this experiment it can be concluded that the rate of regeneration does not depend upon the size, the age (within this range) or whether the animal has been adapted to terrestrial life.

A COMPARISON OF THE RATES OF REGENERATION OF LARVAL AND ADULT AMBLYSTOMA

Since the experiments on Triturus had failed to show any difference in the rates of the various groups considered, experiments were begun on larval Amblystoma jeffersonianum. The larval salamanders in this

experiment were raised in the laboratory from eggs. Some of them were put in a cooler room in order to retard their development; in this manner a series of sizes was provided. Although the room temperature was about the same as it had been for the second experiment with Triturus (about 19°C to 20°C), first fingerbuds of the A. jeffersonianum larvae were observed in one third the time that it had taken the Triturus, and regeneration was more rapid and more perfect. In Table III the larval A. jeffersonianum are grouped in the order of their total lengths, but the length of fore and hind limbs are also given as they indicate the development of the larvae: younger larvae have shorter hind limbs than fore limbs; the adults have longer and larger hind limbs than fore limbs.

TABLE III

COMPARATIVE REGENERATION RATES OF LARVAL A. JEFFERSONIANUM

Animal	Approx. room temp.	Total length in mm	Length of hind limb in mm	Length of fore limb in mm	Meta-morphosis: days after amputation	Number of fingerbuds: days after amputation 13	16
1	20°C	77	14	13	11	0	2
2		67	12	12	18	3	3
3		59	11	11	20	3	3
4		56	10	12	18	3	4
5		49	9	10	34	3	4
6	19°C	48	5	8	not	0	2
7		48	5	8	under	0	2
8		45	6	9	28	0	2
9		44	5	8	days	0	2
10		43	5	8		0	2
11		43	5	8		0	2
12		38	4	7		2	2
13		36	4	6		2	2
14		36	3	6		2	3
15		35	3	6		2	3
16		32	2.5	6		2	4
17		31	2	6		2	3
18		30	.75	5		2	3
19		25	.75	4.5		2	4

From Table III it can be observed that the younger or more undeveloped A. jeffersonianum regenerated more rapidly than the older ones in the same temperature range. Of the five larvae that metamorphosed during the experiment the one which was nearest to metamorphosis regenerated most slowly. In the case of these larvae metamorphosis is gradual up to the time at which they lose their gills.

Their gills disappear rapidly, taking about five days for the whole process. After the disappearance of the gills, metamorphosis continues gradually as adult skin and proportions develop. In Table III metamorphosis refers to the third day of the period during which the gills disappear, because it is at this time that gross changes throughout the body are most rapid. Until this time it is impossible to pick up the larva because its skin is so slimy; after metamorphosis the salamander may be picked up between two fingers as the skin is slightly sticky. The first adult molt may also be observed during the period when the gills are changing.

Controls for this experiment were six larval Amblystoma punctatum and three adults of the same species. The larval A. punctatum got fingerbuds at the same time (13 days after amputation) as did A. jeffersonianum of the same degree of development. The adult A. punctatum were very slow as it was twelve weeks before fingerbuds were visible.

A Comparison of the Rates of Regeneration of Aquatic Larval Newts and Adult Newts

Since there was a difference in the rate of regeneration of newly hatched A. jeffersonianum and A. punctatum an experiment was done on newly hatched and adult Triturus.[2] The salamanders were collected in August in the vicinity of Northampton, therefore it would be expected that some of the larvae would metamorphose into red efts within several weeks. The salamanders were arranged in the following groups.

A. Two larval Triturus, 25 mm and 28 mm long. Neither had dorsolateral spots; they were a yellow green color.

B. Five larvae, 28 mm to 31 mm long. Four were a yellow-green; one was a darker green with the beginning of spots.

C. Three larvae none of which had spots. Two were a yellow-green and 36 mm long. One was tan and 39 mm long.

D. Seven larvae, 37 mm to 41 mm long. They were a general orange-brown color and all but the largest had orange and black spots.

E. Six larvae, 41 mm to 45 mm long. All were an orange-brown with orange and black spots.

F. Three green adults, 80 mm to 85 mm long.

[2]The author wishes to thank Dr. S. Meryl Rose for taking the readings on this experiment and for his advice and encouragement throughout this work.

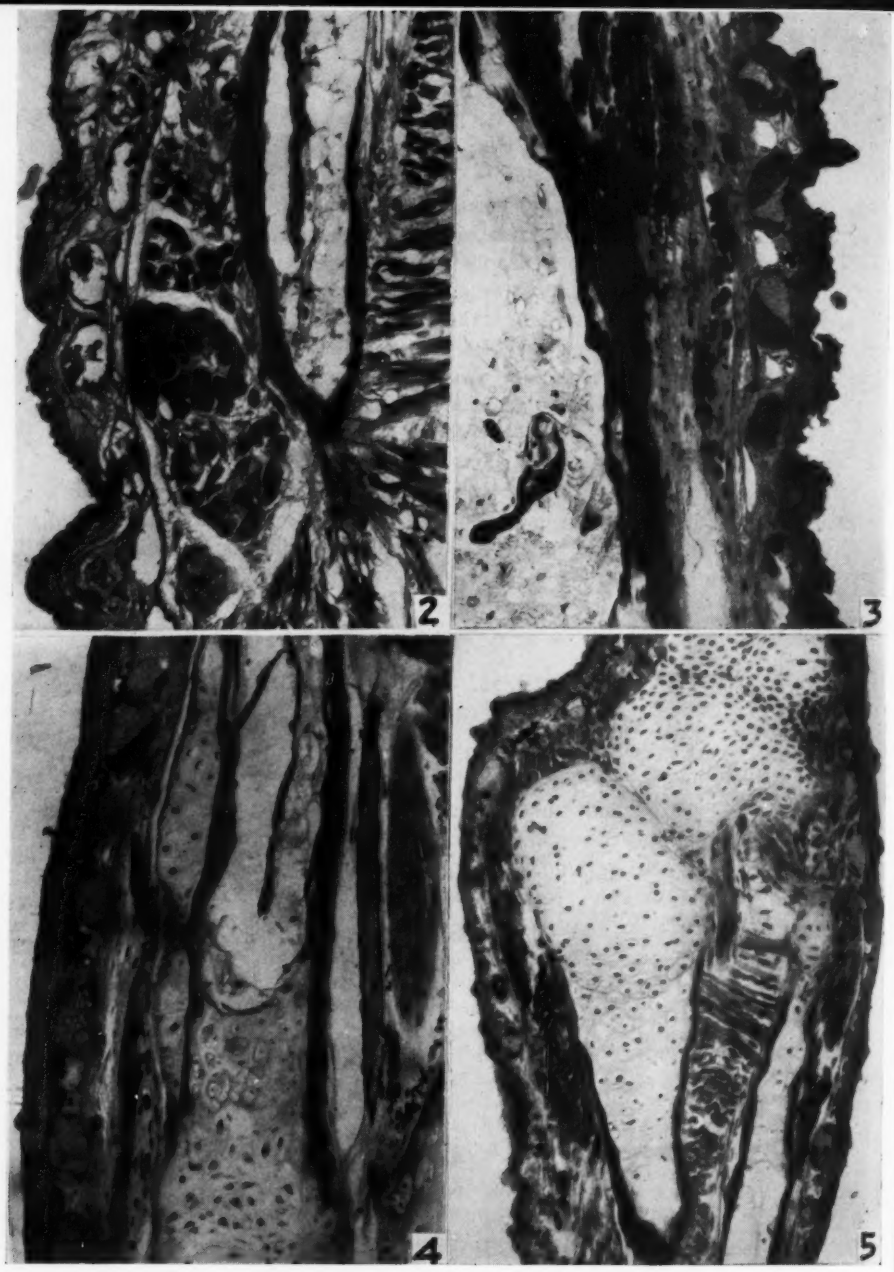

PLATE 1

2. Red eft. A section through a normal wrist showing fully differentiated tissues. X100.

3. Adult Triturus from the vicinity of Northampton. A section through a normal wrist showing bone, muscle, compact dermis, differentiated skin glands and an epidermis many cells thick. X100.

4. Adult Triturus from Wood's Hole. A section through a normal wrist. X100.

5. Larval Triturus from Wood's Hole. A section through a regenerating hand and a normal wrist. The bottom third of this picture is normal tissue. X100.

On the right side there is one large gland similar to those in figures 2, 3 and 4. Salamanders of the types shown in figures 2 to 5 regenerate at approximately the same rate.

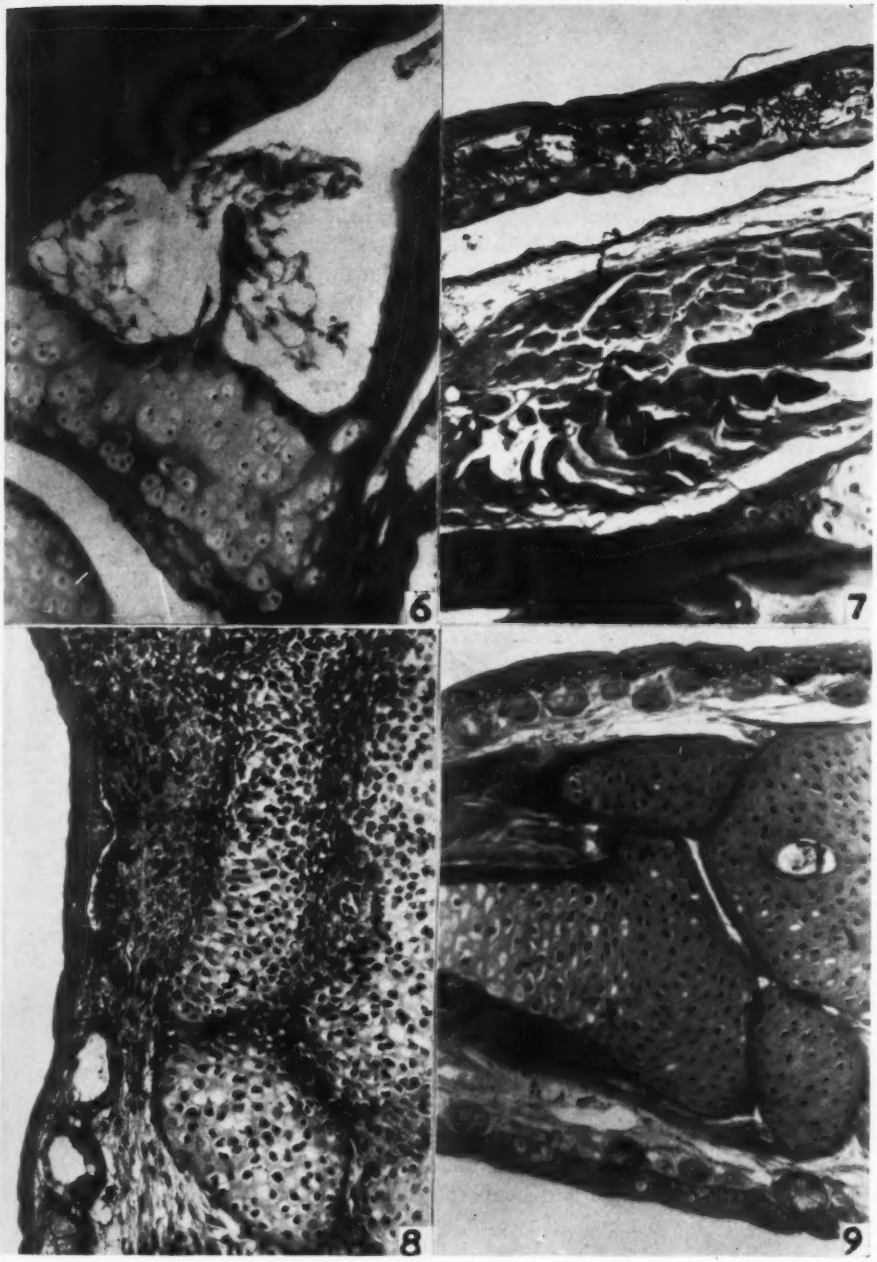

PLATE 2

6. Adult Amblystoma punctatum. A section through normal bone and cartilage at the wrist. X100.

7. Adult Amblystoma punctatum. A section through the skin of the fore arm showing the cutis plate and the complete differentiation of the other structures. X100.

8. Metamorphosing A. jeffersonianum. A section in the region of the wrist. The upper half is regenerating; large glands are forming in the bottom half which is metamorphosing normal tissue. X100.

9. Metamorphosed Triturus. A section through the distal part of the fore arm of one of the larvae of Group E of the last experiment. This is some of the normal tissue which was removed at amputation. Note the adult structure of skin glands and epidermis and the approaching similarity to figure 4. X100.

The results of this experiment are given in Fig. 1. The ordinate represents the days after amputation, and the abscissa represents the various groups beginning with the youngest or least developed which did not metamorphose during the experiment. It is evident that the older the larva is at the time of amputation, the longer it takes it to get four fingerbuds, yet, as shown in the first experiment, after metamorphosis the age of the salamander does not affect the rate of regeneration.

It is also interesting to note that the period of dedifferentiation (from amputation to rounded or cone blastema) is less in young larvae than in older larvae or adults. The rate at which larval and adult Triturus regenerate is dependent (in part) upon the speed of the initial stage of regeneration which is dedifferentiation. Reasons for an increased rate of dedifferentiation in younger larvae will be discussed later.

Histological Comparisons

From the above experiments it is evident that the older the larva was at the time of amputation the longer it took to regenerate. After metamorphosis or certain stages of it in Triturus viridescens there appeared to be no further decrease in the rate of regeneration as the animal grew older. However, there are no data for Amblystoma jeffersonianum and A. punctatum which, after metamorphosis, may regenerate more slowly as they grow older. Histological comparisons were made of the groups of salamanders in order to discover what structural changes could be influencing the rate of regeneration.

From a histological standpoint the salamanders fell into two groups: (1) larval and adult Triturus from Wood's Hole, red eft, adult newts, adult Amblystoma punctatum and metamorphosed A. jeffersonianum; (2) the young larvae of Triturus, A. punctatum and A. jeffersonianum.

Histological studies of the first group show similar structure of skin, cartilage, and bone and are illustrated by figures 2 to 9. It will be noticed that the skin is very well differentiated; there are multicellular glands and the dermal fibers are well developed. The cartilage also is well differentiated and there is ossification of the skeletal elements. Cartilage and bone characteristic of this group are illustrated by fig. 6 which is a section through a fore limb of an adult A. punctatum.

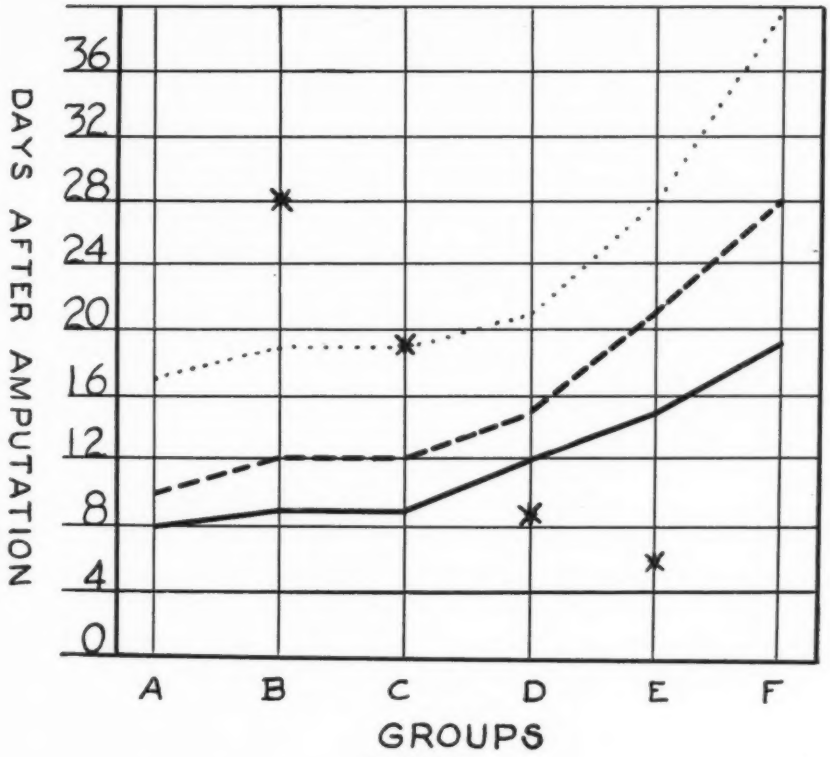

FIGURE 1

A comparison of the rates of regeneration of the various groups of Triturus larvae and adults. The asterisks indicate the day at which the majority of each group had metamorphosed. Group A did not metamorphose during the experiment; Group F had been adults for two or more years. The solid line indicates the average dates for the appearance of a rounded or cone shaped blastema. The broken line indicates the average dates for the appearance of first fingerbuds. The dotted line indicates the average dates for the appearance of four fingerbuds.

On the other hand, the young larval salamanders (group 2) have only incomplete differentiation of skin, cartilage and newly formed bone. Figures 10, 11 and 12 are sections through this larval limb tissue. The skin is only a few cells thick and the glands are unicellular. Where there is a dermis it is very thin and loosely organized.

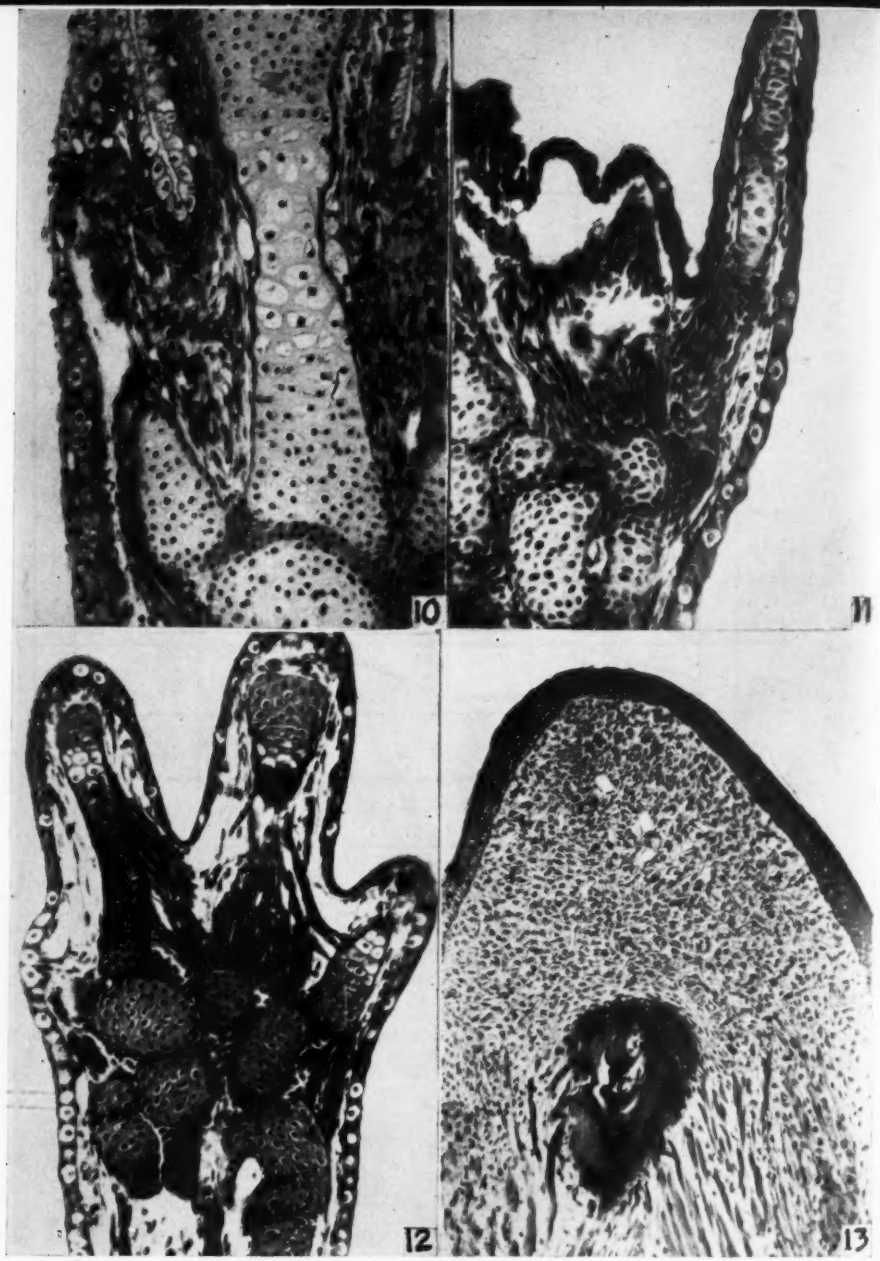

PLATE 3

10. Larval Amblystoma jeffersonianum. A section through a normal hand. Note the embryonic character of the tissues. X100.

11. Larval A. punctatum. A section through a normal hand. X100.

12. Larval Triturus. A section through the amputated hand of one of the larvae in Group A of the last experiment. X100.

Note the similarity of figures 10, 11 and 12; salamanders of this type regenerate rapidly. Note that the epidermis is only two to three cells thick and there is a regular line of Leydig cells. Compare the entirely cartilagenous skeleton of fig. 12 with the skeleton of fig. 9 which had started to ossify.

13. Adult Triturus. Regenerating hand 30 days after amputation. The bottom third is old tissue. X20.

Cartilage and bone are not fully differentiated and still have an embryonic character. It is these young larval salamanders which dedifferentiate more rapidly than the adults whose limbs are more highly differentiated.

Discussion

The foregoing experiments indicate that in Triturus viridescens, A. punctatum and A. jeffersonianum the length of the period of dedifferentiation, which is the initial step of regeneration, is dependent upon the degree of differentiation of the tissues at the time of amputation. In this respect it is interesting to note Polejaiev's (1936) conclusions after amputating through the regenerating portions of tadpole limbs. The newly regenerated limb was less differentiated than the normal limb. If both limbs were amputated at the same level the reamputated limb would regenerate although the normal limb would not. Polejaiev concludes that it is the amount of differentiation which determines whether the limb will regenerate. In these experiments, however, the highly differentiated limbs of adult Amblystoma and Triturus did regenerate. It seems that there are two factors: the degree of differentiation controls the rate of dedifferentiation, and the balance of the various tissues controls the possibility of regeneration.

In the case of frogs (Gidge and Rose, 1944) the dedifferentiation of skin, cartilage and bone does not occur at the same rate, and differentiation occurs while dermis and bone are prominent in the blastema so that their connective tissue and cartilage use up the blastema cells and thus inhibit regeneration. In Triturus all normal tissues have dedifferentiated the same amount (fig. 13) and have equal opportunity to influence the blastema, thus a normal limb is regenerated. But the rate of blastema formation is determined by how quickly the stump can dedifferentiate, and this is determined by how completely the stump tissues are differentiated.

In these experiments there have been cases where adult Triturus of the water stage varied in their rates of regeneration without apparent cause. On close inspection it was observed that the newts which regenerated most quickly had slimy skins while the others had rough or slightly rough skins. But when the experiments were performed for the purpose of comparing their rates it was found that the skin conditions were not permanent. Some light is thrown on this situation

by Richardson (1940) who studied the influence of the anterior pituitary on the regeneration rates of newts. She found that anterior pituitary increased the rate of regeneration; it also gave the newts slimy skins and regenerants were inclined to be abnormal and flexed ventrally. These same characteristics were observed in some of the more quickly regenerating newts of these experiments and may have been caused by varying amounts of anterior pituitary. There are differences in the regeneration rates of adult newts and other salamanders which need further investigation, but they are minor differences when compared with the rates of young larvae. For instance, a slimy newt may get fingerbuds in five weeks, when a rough skinned newt would take six weeks; but under the same conditions a young larval newt may have fingerbuds within a week and a half.

After dedifferentiation is complete and differentiation has started there may be many factors such as hormones and metabolism which govern the rate of growth, but the rate of dedifferentiation is dependent upon the degree of differentiation.

Summary

1. It is generally accepted that young salamanders regenerate more rapidly than old ones. Experiments were performed on Triturus viridescens, Amblystoma punctatum and A. jeffersonianum to determine what caused the difference in rate.

2. From experiments on adult Triturus, red efts and neotenous newts it was concluded that the rate of their regeneration was not affected by a terrestrial or aquatic habit, size or age after metamorphosis.

3. Experiments with young larvae of Triturus, A. punctatum and A. jeffersonianum indicate that there is a gradual decrease of the rate of regeneration as amputations are performed later in larval life. After metamorphosis young and old Triturus regenerate at the same rate.

4. Histological studies show that the newly hatched larvae have incompletely differentiated tissues so that it is relatively easy for them to dedifferentiate and form a blastema, while the adults of Triturus, A. punctatum and A. jeffersonianum, because of their highly differentiated tissues, take a long time to form a blastema.

5. It is concluded that there are two factors governing regeneration: (1) the degree of differentiation of the tissues and (2) the balance of the tissues after dedifferentiation. The former determines the rate of differentiation (and in this manner affects the total time necessary for regeneration) and the latter factor determines whether regeneration will be normal, abnormal, or will not occur.

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